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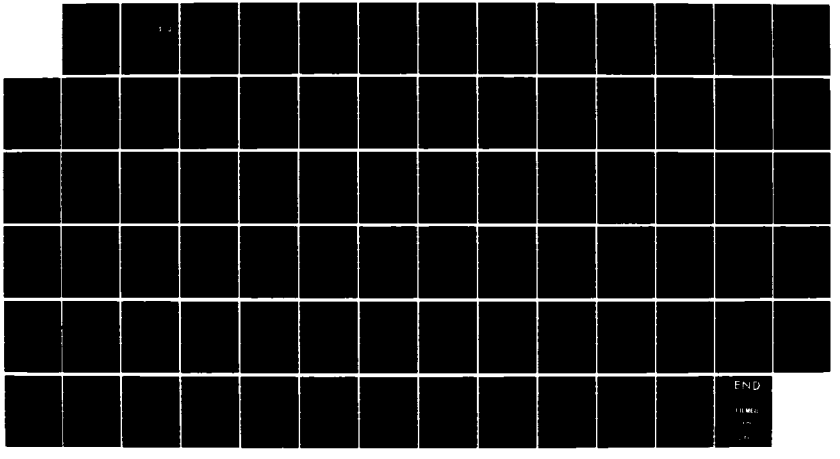

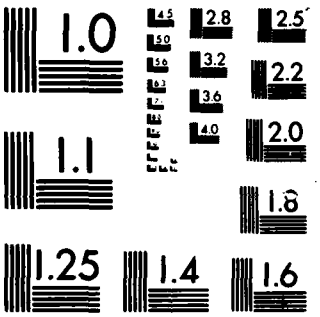

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TOXICITY OF TNT WASTEWATERS TO AQUATIC ORGANISMS

Final Report

Volume III

Chronic Toxicity of LAP Wastewater and 2,4,6-Trinitrotoluene

by

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TNT	Fathead minnow	Ictalurus punctatus
LAP	Daphnia magna	Early life stage studies
2,4,6-trinitrotoluene	Chronic studies	Channel catfish
RDX	Pimephales promelas	Rainbow trout
Hexahydro-1,3,5-trinitro-1,3,5-triazine		Water quality criteria
		Salmo gairdnerii

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Early life stage tests were performed with LAP water (a 1.6:1 mixture of TNT and RDX) and TNT using rainbow trout, fathead minnows, and channel catfish as test organisms. Chronic toxicity studies were performed with TNT and LAP water using fathead minnows and Daphnia magna as test organisms. A chronic study was also performed with irradiated LAP water using Daphnia magna. Based on the data from these studies as well as prior acute studies, water quality criteria based on US EPA-recommended procedures were developed for LAP water

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20. ABSTRACT (Concluded)

and TNT. For both LAP water and TNT, concentrations of 1.3 and 0.9 mg/L, respectively, should not be exceeded in a 24-hour period. For LAP water, 0.19 mg/L should be considered the 24-hour average allowable concentration. For TNT, a concentration of 0.04 mg/L should be used as an interim 24-hour average allowable concentration until a chronic no-effect level is experimentally defined for fathead minnows exposed to TNT.

FOREWORD

The U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD, is conducting a research program for the purpose of developing the scientific data base necessary for assessing the potential environmental hazards associated with compounds unique to the munitions industry. From these data, criteria will be developed that are qualitative or quantitative estimates of the concentrations of a pollutant in ambient waters that, if not exceeded, should ensure the protection of aquatic organisms and human health. When compared with actual or estimated environmental concentrations, these criteria will form the basis of a hazard assessment. In addition, these criteria will be used to assess the adequacy of current pollution abatement technologies and thus influence research and development in this area.

This report represents a portion of the data base being developed on TNT and its associated wastewaters and should not be construed as a complete evaluation or as official policy of the U.S. Army Surgeon General.

This work was conducted under the technical control and review of the U.S. Army Medical Bioengineering Research and Development Laboratory: J. Gareth Pearson and William H. van der Schalie (Aquatic Toxicology), Jesse J. Barkley, Jr. (Analytical Chemistry), and Jerry W. Highfill (Statistical Analysis).

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EXECUTIVE SUMMARY

This report is the third volume in a series of four reports on the toxicity of trinitrotoluene (TNT) wastewaters to aquatic organisms. The information presented in these reports was developed in a multiphase study performed by SRI International for the U.S. Army Medical Research and Development Command (USAMRDC) under Contract No. DAMD17-75-C-5056. The study was undertaken to assist USAMRDC in developing a data base for assessing the potential hazards to aquatic life of wastewaters from TNT manufacturing and processing plants.

This report presents and discusses the results of early life stage and chronic studies with TNT, LAP water, and photolyzed LAP water. LAP water is a synthetic wastewater composed of TNT and RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) mixed in a ratio of 1.6 to 1. This ratio is based on chemical analysis and characterization of the authentic wastewater produced by load, assemble, and pack (LAP) facilities that handle an explosive mixture known as Composition B.

Based on the results of these studies, water quality criteria for LAP and TNT were calculated using EPA recommended procedures (EPA 1979). The criteria are composed of two parts; one is a concentration that cannot be exceeded in a 24-hour period, and the other is an average allowable concentration for a 24-hour period. For LAP, the 24-hour maximum concentration was calculated to be 1.3 mg/L, and the 24-hour average concentration was calculated to be 0.19 mg/L. For TNT, the maximum acceptable concentration was calculated to be 0.94 mg/L. Because toxic effects occurred in the fathead minnow chronic study at the lowest TNT concentration tested (0.04 mg/L), it was not possible to experimentally define a chronic no-effect level. By arbitrarily defining 0.04 mg/L as the end point for the fathead minnow study, a 24-hour average allowable concentration of 0.04 mg/L was calculated. It is suggested that this level be considered an interim value until a chronic no-effect level can be experimentally determined.

Early life stage studies were conducted on TNT and LAP water with rainbow trout, channel catfish, and fathead minnows. Full life-cycle chronic studies were performed on TNT and LAP water with fathead minnows and Daphnia magna and on irradiated LAP water with D. magna. For TNT, the effect/no-effect concentrations from the chronic studies were < 0.04 mg/L for fathead minnows and 1.03 and 0.48 mg/L for D. magna. For LAP water, the effect/no-effect concentrations for fathead minnows were 0.62 and 0.28 mg/L, while the results for daphnids were ambiguous. Irradiated LAP water was similarly or slightly less toxic to daphnids than nonirradiated LAP water. In comparing these results to those from earlier acute studies, it appears that TNT caused chronic effects at concentrations 20 to 40 times less than concentrations that caused acute effects in fish. Similarly, LAP water produced chronic effects at concentrations up to 10 times less than those that resulted in acute effects in fish.

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INTRODUCTION

The production of munitions compounds generates a significant volume of wastewaters, which have historically been discharged into the environment with little or no treatment. To assess the hazard of these wastewaters to human health and to aquatic life, the U.S. Army Medical Research and Development Command (USAMRDC) has funded a comprehensive investigation to develop a scientific data base comprising data from literature reviews, on-site field studies, and laboratory investigations in mammalian and aquatic toxicology.

Of the various kinds of wastewaters produced in the manufacture and processing of munitions compounds, condensate and LAP wastewaters are of major concern to the USAMRDC. Condensate wastewater is produced during the continuous process for manufacturing 2,4,6-trinitrotoluene (TNT) and comprises at least 30 organic compounds, with 2,4-dinitrotoluene (DNT) accounting for almost 50% of the total dissolved organics (Spanggord et al., 1978). LAP wastewater is produced at load, assemble, and pack (LAP) facilities during the washing of shells and other equipment. The composition of LAP wastewater depends on the particular kind of explosive formulation being processed by the LAP facility. However, the LAP wastewater of primary concern is produced by LAP facilities handling an explosive formulation called Composition B (COMP B) and is composed primarily of TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX).

Under Contract DAMD17-75-C-5056, SRI International conducted a laboratory study to determine the acute, subchronic, and chronic toxicity to aquatic organisms of condensate wastewater, of LAP wastewater from COMP B processing plants, and of selected organic components of both wastewaters. The study comprised four phases, each with several tasks, and followed the approach proposed by Pearson and co-workers (1979) for toxicologic evaluation of complex industrial wastewaters.

The results of SRI's study are presented in a series of four reports. This report is the third volume in the series; it presents and discusses the methodologies and results of early life stage and chronic studies performed with aquatic organisms and TNT, synthetic LAP wastewater (LAP water)--a 1.6:1 blend of TNT and RDX--and photolyzed LAP water (LAP-Irrad). The Appendix to this volume contains graphic displays of the data from each test (Bailey et al., 1984).

The other reports in the series are: Volume I, "Acute Toxicity of LAP Wastewater and 2,4,6-Trinitrotoluene" (Liu et al., 1984), which describes the overall testing approach and the facilities, equipment, and procedures used to conduct acute toxicity and bioconcentration studies on TNT, LAP, and related materials; Volume II (Liu et al., 1984), "Acute Toxicity of Condensate Wastewater and 2,4-Dinitrotoluene"; and Volume IV (Bailey et al., 1984), "Chronic Toxicity of Condensate Wastewater and 2,4-Dinitrotoluene".

MATERIALS AND EQUIPMENT

Test Materials

The substances tested in this study were synthetic LAP wastewater (LAP water), 2,4,6-trinitrotoluene (TNT), and photolyzed LAP water (LAP-Irrad). The sources and purities of the test materials and the method used to irradiate LAP are described in Volume I (Liu et al., 1984).

Test Organisms

The following species of fish and invertebrates were used:

Fathead minnow (Pimephales promelas)
Channel catfish (Ictalurus punctatus)
Rainbow trout (Salmo gairdneri)
Water flea (Daphnia magna).

These species were selected because they represent a range of taxonomic groups that have different habitat requirements and also exhibit varying sensitivities to chemical toxicants. Consequently, it was felt that water quality criteria based on tests with these species should afford a reasonable degree of protection for most, if not all, species undergoing exposure to munitions wastewaters. In addition, test methods for the selected species were fairly well established, which increased the probability of successfully conducting the laboratory exposures.

Fathead minnows and Daphnia magna were obtained from SRI's breeding colonies. The breeding stocks were reared under flow-through conditions at 20 and 25°C for the daphnids and minnows, respectively. The adult minnows were fed frozen adult brine shrimp (San Francisco Bay Brand, Newark, CA), live daphnids, and trout chow (Clark's Feed Company, Salt Lake City, UT). Adult daphnids were fed Selenastrum capricornutum alone or in conjunction with a vitamin supplement (Goulden et al., 1982). The photoperiod was set at 16 hours light (100 ft candles) and 8 hours dark. Channel catfish eggs were obtained from Alex Fish Company, San Rafael, CA, and rainbow trout eggs were obtained from the Mt. Lassen Trout Farm, Red Bluff, CA.

Diluent Water

We used dechlorinated tap water to culture and maintain the test animals, to prepare the stock solutions, and as the diluent water for the flow-through tests. The water was dechlorinated by passing it through a series of columns, each containing 0.042 m^3 of activated carbon that was renewed every 3 months by a local water purification firm (Culligan, Santa Clara, CA).

The laboratory tap water is a blend from the Hetch Hetchy, Calaveras, and San Antonio Reservoirs. On the average, about 75% of the water originates from the Hetch Hetchy Reservoir, which is located in the Sierra Nevada. From late spring through the fall, about 95% of the water comes from Hetch Hetchy Reservoir. During the winter and early spring before the snow begins to melt, the blend is composed primarily of water from the two low-elevation reservoirs.

The San Francisco Water Department (SFWD) annually analyzes the water from these reservoirs for various minerals and other constituents. Table 1 presents the average and range for each of the 43 parameters measured by the SFWD during the periods 1969 to 1971 and 1975 to 1978 in water samples from the three reservoirs.

We performed a less comprehensive analysis of our dechlorinated tap water in 1975. The results are presented in Table 2. In 1978, we began analyzing the dechlorinated tap water routinely for hardness, alkalinity, acidity, pH, conductivity, and residual chlorine. Table 3 presents the average, standard deviation, and range for each of these parameters during the study period.

Over the seven years that our aquatic toxicology facility has been in operation, our dechlorinated tap water has been satisfactory for rearing and maintaining a variety of aquatic animals. However, during Phases I to III of this study, we experienced intermittent problems with unacceptably high control mortality (>20%) during tests with daphnids. When this occurred, we repeated the tests until acceptable results were obtained. The problem was later determined to be caused by seasonal fluctuations in the hardness of the diluent water. Depending on the mixture of waters obtained from the three storage reservoirs, hardness dropped to levels as low as 15 mg/L (as $CaCO_3$). The periods in which the laboratory received very soft water were found to correspond to the periods in which we observed poor daphnid survival. To ensure that these fluctuations in the hardness of the diluent water did not adversely affect the results of the daphnid chronic studies, a solution of magnesium, calcium, and potassium salts (Marking and Dawson, 1975) was metered into each diluter at a rate sufficient to maintain a minimum hardness of 35 to 40 mg/L.

Temperature Control

To maintain the temperature of the exposure chambers at the desired level, both the water and room temperatures were controlled. Room temperatures were controlled by thermostatically controlled heat pumps set at the desired test temperature. In tests requiring heated diluent water, a thermostatically controlled 2000-W stainless-steel immersion heater was used to adjust the temperature of the incoming water in head tanks before the water entered the diluter. In tests requiring water at lower-than-ambient temperatures, chilled water was supplied to a head tank by a 9000-BTU water chiller at the approximate test temperature and was then maintained at the desired temperature by a thermostatically controlled 2000-W immersion heater.

Table 1. WATER QUALITY CHARACTERISTICS OF WATER FROM HETCH HETCHY, SAN ANTONIO, AND CALAVERAS RESERVOIRS

Measured Parameter	Hetch Hetchy			San Antonio			Calaveras		
	Mean[a]	Range	Nb	Mean[a]	Range	Nb	Mean[a]	Range	Nb
Calcium	1.08	0.3-1.6	7	31.7	23.4-50.5	4	29.3	25.7-34.5	3
Magnesium	0.42	0.0-1.8	7	12.0	8.6-15.1	4	10.4	7.3-13.1	3
Sodium	0.89	0.5-1.3	7	20.9	15.5-30.0	4	9.7	8.5-11.0	3
Potassium	0.34	0.2-0.6	7	2.2	2.0-2.4	4	1.6	0.9-2	3
Bicarbonate	6.0	2.6-9.2	7	131.6	106.6-146.4	4	114	89-146.4	3
Carbonate	0.0	0.0-<0.5	7	3.6	0-9.6	4	0.4	0-1.1	3
Carbonic Acid	2.25	1.9-2.6	2	43	0-86	2	72	-	1
Chloride	0.09	0.1-0.5	7	29.2	13.5-52.1	4	9.5	8-10.5	3
Sulfate	0.51	0.25-1.3	7	26.4	21.8-31.9	4	22.5	18.0-28.3	3
Fluoride	0.008	0.02-0.03	6	0.098	0-0.17	4	0.1	0.1-0.15	3
Aluminum	0.02	0.01-0.05	7	0.035	0.01-0.06	4	0.04	0.01-0.07	3
Arsenic	0.02	<0.001-0.037	2	<0.01	-	4	<0.01	-	3
Barium	<0.5	-	7	<0.5	-	4	<0.5	-	3
Cadmium	<0.002	-	7	<0.002	-	4	<0.002	-	3
Chromium	<0.01	-	7	<0.01	-	4	<0.005	-	3
Copper	0.005	0.0-0.01	4	0.5	<0.01-0.01	4	0.045	<0.01-0.06	2
Iron	0.06	0.005-0.12	5	0.08	<0.01-0.14	3	0.25	<0.01-0.25	1
Lead	<0.05	-	7	<0.05	-	4	<0.02	-	3
Manganese	<0.02	-	7	<0.02	-	4	<0.01	-	3
Selenium	<0.01	-	6	<0.01	-	4	<0.01	-	3
Silver	<0.03	-	7	<0.06	-	4	<0.005	-	3
Zinc	0.04	<0.01-0.06	3	0.016	0.009-0.03	3	0.012	0.003-0.02	2
Silica	3.5	0.2-6.4	7	3.7	0.2-7.0	4	7.9	3-10.5	3
Ammonia	0.03	0.02-0.05	5	0.15	0.02-0.214	2	0.115	0.1-0.13	2
Ammonia (free)	0.05	-	7	0.05	-	4	0.03	0.02-0.04	2
Boron	0.03	0.006-0.07	7	0.17	0.005-0.25	4	0.075	0.025-0.13	3
Cyanide	0.01	-	6	0.01	-	3	0.001	-	2
Nitrate	0.5	-	7	1.3	0.05-2.9	3	2.4	0.05-2.4	1
Nitrite	-	0.001-0.004	7	0.014	0.001-0.014	1	0.003	0.001-0.003	1
Phosphate	0.01	0-0.025	3	0.11		3	0.05	0.04-0.08	3
Tannins & Lignins	0.06	0.05-0.1	4	0.075	0.01-0.25	2	0.05	0.05-0.05	1
Dissolved oxygen	8.6	7.6-10.3	6	7.8	0.05-0.1	3	9.5	9.1-10.1	3
Total apparent ABS	0.05	-	7	0.05	5.9-9.0	4	0.05	-	3
Hardness (as $CaCO_3$)	3.6	3.0-4.6	7	128.4	-	4	116	99.0-130	3
Alkalinity (as $CaCO_3$)	5.1	2.6-7.5	7	107.5	94-163	4	98.8	87.3-120	3
Total solids	11.0	6.9-17	7	206.8	71-143	4	162.3	155-170	3
Conductivity (µmhos/cm)	14.3	8-20	7	334.8	196-216	4	262.3	252-283	3
pH (units)	7.1	7.0-7.2	7	8.1	259-368	4	8.1	7.9-8.2	3
Turbidity (units)	0.89	0.11-4	7	2.4	7.6-8.8	4	2.4	0.3-6.4	3
Color (units)	1.6	0-5	7	5.0	0.1-6	4	23.3	0-50	3
C chloroform extract (µg/L)	-	-	7	85.6	0-10	1	-	-	-
Radioactivity (µci/g)									
Alpha	0.43	0.3-0.6	3	0.6	-	1	-	-	-
Beta	1.8	0.6-3	2	7	-	1	-	-	-

[a] mg/L except where marked otherwise.
[b] Number of years the specified parameter was measured.

Table 2. CHEMICAL ANALYSIS OF SRI DECHLORINATED TAP WATER (1975)

Analysis	Concentration (mg/L)
Calcium (as Ca)	8.4
Magnesium (as Mg)	2.5
Potassium (as K)	0.40
Sulfate (as SO_4)	9.2
Nitrate (as NO_3-N)	<0.005
Nitrite (as NO_2-N)	0.001
Free ammonia	0.060
Organic ammonia	0.375
Phenol	<0.001
Residual chlorine	<0.003
Chloride	4.04
Fluoride	0.30
Cyanide	<0.01
Iron	0.08
Copper	0.0041
Zinc	0.0026
Cadmium	0.0012
Chromium	0.008
Nickel	<0.050
Lead	0.0007
Total alkalinity (as $CaCO_3$)	23.3
Total hardness (as $CaCO_3$)	31.2
Total dissolved solids	48.0

Table 3. MEAN, RANGE, AND STANDARD DEVIATION OF WATER QUALITY PARAMETERS MEASURED ROUTINELY IN SRI'S DECHLORINATED TAP WATER

Period: May 1978 to May 1979

Parameter	Units	Mean	S.D.	Range	Number of Analyses
Hardness	mg/L $CaCO_3$	32.4	22.4	7-123	90
Alkalinity	mg/L $CaCO_3$	30.8	18.1	10-110	91
Acidity	mg/L $CaCO_3$	6.0	3.6	5-22.5	34[a]
pH	—	8.1	0.5	6.6-9.0	90
Conductivity	μmhos/cm	82.0	51.4	28-263	87
Total residual chlorine	μg/L	1.9	1.2	0.1-7.0	195[b]

[a]57 values below detectable limits (< 5 mg/L).

[b]Chlorinity tester inoperative on 25 of the 91 days water quality was tested. Value was below detectable limits 4 times (< 0.001 mg/L).

Toxicant Dilution Equipment

In the early life stage studies on catfish and trout, we used a toxicant dilution system developed at SRI for conducting short-term tests. The system is composed of two constant-head reservoirs, one located above the other. The upper reservoir, measuring approximately 15 x 5 x 183 cm (H x W x L), was used to deliver stock solutions of the toxicant. The bottom reservoir, measuring approximately 22 x 15 x 365 cm (H x W x L), was used to deliver water.

The water reservoir was equipped with 24 adjustable flow meters capable of measuring flows up to 300 mL per minute. Water was pumped to this reservoir from a secondary reservoir equipped with temperature-control and aeration devices and connected to the laboratory water supply through a float valve. Excess water in the primary reservoir was returned to the secondary reservoir by gravity. The toxicant stock solution was recirculated by pump between the toxicant reservoir and a 55-gallon polyethylene-lined steel drum in which the toxicant stock was prepared. The toxicant was metered by Teflon capillary tubes, and the delivery rate was adjusted by increasing or decreasing the vertical distance of the distal end of the tube from the level of the liquid in the toxicant reservoir.

The toxicant stock solution and water were delivered to a mixing chamber that divided the total volume equally between two exposure chambers. After the flows of toxicant and water necessary to obtain each desired toxicant concentration were calculated, the flows were set, using a graduated cylinder and stopwatch.

Early life stage studies with fathead minnows and chronic studies with fathead minnows and D. magna were conducted using Mount-Brungs style diluters (Mount and Brungs, 1967). Each diluter delivered approximately 500 mL of exposure solution to each mixing cell, where it was split into two 250-mL volumes and delivered to each of two duplicate aquaria. During the early rearing stage, when the fathead minnow fry were being reared in duplicate rearing chambers, the 250-mL volume was split again so that each chamber received approximately 125 mL.

The delivery rates of the Mount-Brungs diluters were controlled by regulating the water flow into the diluter with a valve during the tests with fathead minnows. Because much lower flows were required for the daphnid studies, the cycling rate of each diluter was controlled by a capillary tube to drain the bucket that operated a microswitch controlling the incoming water. By slowing the flow rate from the bucket, the time interval between cycles was increased, thereby reducing the overall delivery rate.

The toxicant was usually delivered to the diluters from 55-gal polyethylene-lined steel drums. In the fathead minnow tests, a recirculating system incorporating a toxicant head tank behind and slightly above the main mixing cell was used. A capillary tube drained the toxicant from the head-tank into a calibrated beaker or graduated cylinder containing a glass

siphon connected to the water outflow from the W-1 cell. When the W-1 cell started to drain, a vacuum started the siphon from the beaker containing the toxicant, and both diluent and toxicant entered the mixing cell. The amount of material in the beaker could be varied by raising or lowering the beaker in relation to the toxicant headtank. In addition, the beaker contained a drain to prevent overfilling in case the diluter cycling rate slowed or stopped. The toxicant was delivered directly into the main mixing cell of the diluters used in the daphnid chronic studies by Mariotte bottles filled from the stock barrels or by metering pumps directly from the stock barrels. All Mount-Brungs diluters were equipped with counters to monitor the cycling rate and to aid in ensuring the proper function of the diluters.

METHODS

Toxicity Testing

General

Tests were performed in duplicate with six treatment levels including the controls. Chronic tests were performed in 30.5 x 91.4 x 30.5 cm (H x L x W) glass aquaria containing approximately 40 L of water for the fathead minnow tests and 28 L of water for the daphnid tests. Early life stage studies were performed in 19-L aquaria containing 15 L of test solution. Fathead minnow and channel catfish eggs were exposed in eggs cups made from 5-cm diameter glass or PVC tubing with one end covered with 200-μ Nitex screen. Fathead minnow fry hatched in the egg cups were transferred to rearing chambers (30 x 30 x 5 cm, H x L x W) constructed from glass except for the front panels, which were made from Nitex screen (200 μ) to allow passage of water through the chambers. Channel catfish fry were transferred directly to the aquaria. Eggs from rainbow trout were simply placed on the bottoms of the aquaria during exposure.

The locations of the test aquaria were randomized within each replicate series. Nominal test temperatures were 12°C for the trout early life stage studies, 25°C for the catfish early life stage studies, 25°C for the fathead minnow early life stage studies, 25°C for the fathead minnow chronic studies, and 20°C for the daphnid chronic studies. Diluter flow rates were set to provide a minimum of four tank volumes per day; this rate was increased as necessary to maintain water quality and/or desired chemical concentrations. A photoperiod of 16 hours light and 8 hours dark was used for all tests except the fathead minnow chronic studies, which used an EPA-recommended variable photoperiod corresponding to that of Evansville, IN (EPA, 1972). At the start of each series, this photoperiod corresponded to 1 December and was adjusted as appropriate at 2-week intervals.

Early Life Stage Studies

Channel Catfish. These tests were scheduled to be initiated with 30 eggs per treatment level in each of the duplicate test series. However, we encountered difficulties separating the eggs because they were past the initial hardening stage and were easily damaged when handled. Therefore, we cut off similar-sized clumps of eggs from the original egg masses, counted the eggs in each mass, weighed them, and transferred them into the egg cups.

A problem with using this approach was that, because the eggs were not separate, the fungus-affected eggs could not be removed to prevent the spread of disease to other eggs. To minimize problems with fungal infection, we flushed the eggs daily with malachite green up to the time of

hatching (Leitritz and Lewis, 1976). After hatching, the fry were transferred from the egg cups into the aquaria for a 30-day post-hatch exposure period. During this period, the fry were fed brine shrimp nauplii (cysts obtained from San Francisco Bay Brand, Newark, CA), frozen adult brine shrimp, and dried trout chow ad libitum three times per day. Excess food and waste materials were siphoned from the bottom of the tanks as necessary. The surviving fry were measured (total length) at the end of the exposure period. Chemical concentrations were determined twice weekly, alternating between the replicates.

Fathead Minnows. These tests were initiated with 30 embryos (24 hours old) per egg cup. Two egg cups were used per tank. The tests were generally terminated 30 days after initiation and, with the exception of the test on condensate, they were performed in duplicate. After hatching, the fry were counted and transferred into larval rearing chambers. During the post-hatch exposure period, the fry were fed brine shrimp nauplii three times daily. Excess food and waste materials were siphoned from the bottom of the tanks as necessary. Total fry length was determined photographically or by direct measurement at the end of the exposure period.

Chemical concentrations were routinely determined prior to initiating the test and weekly thereafter, alternating between replicates.

Rainbow Trout. These tests were initiated with 60 eggs per duplicate tank at each exposure level. The eggs were fertilized in the presence of the toxicant and allowed to water-harden before they were transported to the laboratory. In the first series of tests, a different female was used for each concentration; in the second test series, eggs from different females were randomized over the mixing containers before they were fertilized. Because a low overall fertility was observed in one of the tests in the second series, the test was restarted using eyed eggs obtained directly from the hatchery. The first series of tests were terminated 30 days after hatching was completed, and the second series were terminated after a 60-day post-hatch exposure period. Once the fry entered the swim-up stage, they were fed a combination of Artemia nauplii, dry trout food, and frozen adult brine shrimp three times per day. Throughout the tests, the tanks were inspected daily and dead eggs and fry were removed. At the end of the test, surviving fry were anesthetized with MS-222 and individually weighed and measured (total length).

Chemical concentrations were determined weekly, alternating between the replicates.

Chronic Studies

Fathead Minnows. These tests were initiated by randomly distributing a minimum of 40 eggs to each of two egg cups suspended in each tank. The duplicate series were started approximately 1 week apart. During the period of embryo development, the egg cups were inspected daily and dead eggs were removed. Once the fry began to hatch, the cups were not disturbed except for daily checks to determine whether hatching had been

completed. If the hatching process took longer than 24 hours to complete, brine shrimp nauplii were added to the egg cup twice daily to ensure a food source for the hatched fry. When hatching was completed in all cups, deformed and normal fry were counted, and the normal fry were transferred to rearing chambers.

Fry were maintained in the rearing chambers for 90 days. During this period they were fed a mixture of brine shrimp nauplii, dry trout food, and frozen adult brine shrimp four times per day. The food mix varied, depending on the size of the fry. On Days 30, 60, and 90 post-hatch, the rearing chambers were removed from the tanks, placed on millimeter grid paper and photographed several times. These pictures were used to determine the total length of the fry during this period as well as provide an indication of fry survival. After the pictures were taken at 90 days, the fry were released from the rearing chambers into the aquaria. After release, we continued to observe the fry for signs of developing breeding characteristics in the males, such as dark banding, blunt snouts, and tubercles.

When several males were obvious in all of the tanks, the fish were removed from the tanks and carefully segregated according to sex. Males were determined on the basis of banding, blunt snouts, or tubercles. Females were differentiated by the presence of urogenital papillae. A third category was reserved for fry whose sex we could not readily determine. Four males and four females were then selected, weighed and measured, and randomly assigned as individual pairs in stainless-steel breeding cages located in the lower two-thirds of each tank. The remaining fish were anesthetized, weighed and measured, and preserved in 10% formalin according to sex.

Spawning substrates were made by coating the inside of a semicircular piece of PVC pipe with silicone sealant and embedding fine sand into the silicone sealant before it hardened. The pieces of PVC pipe were formed by cutting a 4-in. diameter Schedule 40 pipe in half lengthwise and then cutting each half into 3-in. sections. After the silicone sealant had dried, the excess sand was brushed off and the substrates were soaked for 24 hours in dechlorinated tap water. One substrate was added to each breeding chamber.

Substrates were inspected daily for eggs. If eggs were present, the substrate was replaced with a clean one and placed under a low-power microscope where the eggs could be removed and counted. A minimum of 35 eggs were selected at random and placed in an egg cup to determine hatchability. If the spawn did not contain at least 35 eggs, the eggs were counted and discarded. After the eggs hatched, the fry were counted and discarded or added to a rearing chamber if one was available. We attempted to rear two batches of F_1 fry to 30 days and two batches to 60 days in each tank. If possible, each set of fry selected for rearing was obtained from a different spawning pair.

In addition to hatchability and mortality, records of deformities and total lengths and weights were taken for the F_1 fry. Lengths and weights were taken at the end of the exposure period, and interim 30-day lengths were determined photographically on fry reared to 60 days.

Each test was terminated when no spawns occurred in any concentration for 1 week. The following data were prepared for statistical analysis:

F_0 Survival

Egg
30-day fry
60-day fry
90-day fry
120-day fry
150-day fry
178-day fry

F_0 Growth

30-day length
60-day length
90-day length

F_0 Fry Deformities

F_0 Fertility Measurements

Breeding pair survival
Spawns per pair
Eggs per spawn
Eggs per pair per day
Eggs per pair

F_1 Survival

Egg
30-day
60-day

F_1 Deformities

F_1 Growth

30-day length
30-day weight
60-day length
60-day weight

Global Indices

F_0 90-day cumulative fry survival
F_0 178-day cumulative fry survival
F_0 60-day biomass
F_1 60-day cumulative fry survival
F_1 60-day standing crop
Total survivability index
Total productivity index

Daphnia magna. These tests were conducted in 80-L aquaria that containing approximately 28 L of water. The daphnids were housed in 400-ml beakers, each having a 2-cm-wide by 5-cm-long hole cut in the side. This hole was covered with 200-μ Nitex screen. Ten beakers were placed in each aquarium; seven of the 10 beakers received one daphnid each and the remaining three beakers received five daphnids each.

Young daphnids were reared in a colony maintained under the same conditions as the tests, except that individual beakers contained two adults each. Reproduction was carefully monitored in the colony to ensure that daphnids used in the tests did not come from the first brood. Twenty-four hours before a test was scheduled to begin, all young were removed from the beakers in the rearing colony. On the day of the test, the new young were removed from the beakers, pooled, and distributed randomly into the test beakers using a large bore pipet. This procedure was repeated the following day to start the replicate series. If a sufficient number of young was

not available to initiate a test, this procedure was repeated until enough could be obtained within a 24-hour period.

The beakers were inspected daily for mortality and young. Dead daphnids were removed, and young were removed and counted. The daphnids exposed to TNT were fed algae (Selenastrum capricornutum) twice daily at the rate of 30,000 cells/mL. In the test on LAP (Series A), the algal diet was supplemented with a daphnid formula made from yeast, trout chow, and dried alfalfa to increase reproduction (Biesinger and Christensen, 1972). Although young production did increase, mortality also increased. As a result, a diet of algae supplemented with the vitamin mix recommended by Goulden et al. (1982) was given to daphnids in the tests on LAP (Series B) and irradiated LAP. The tests were terminated after 28 days of exposure, and surviving daphnids from the beakers that contained one individual were measured with an ocular micrometer. Data obtained from each test included:

(1) Mortality at 7, 14, 21, and 28 days.
(2) Total reproduction at 7, 14, 21, and 28 days.
(3) Young produced per female at 7, 14, 21, and 28 days.
(4) Young produced per female per reproductive day.
(5) Length (to base of spine) at 28 days.
(6) Days until first young produced.

Chemical Analyses

Chemical concentrations were measured weekly in each treatment level, alternating between the replicates. Stock concentrations were determined before the stocks were added to the toxicant reservoirs. Twenty-four hours after the new stocks were added to the diluter, a sample was taken from the highest concentration to ensure that the proper concentrations were being delivered. In the event of a diluter malfunction, samples were taken from all concentrations to determine the extent to which the malfunction affected the test concentrations. Samples were also taken 24 hours after the malfunction was corrected to verify that the diluter was again working properly. Analytical methods are described in detail in Volume I (Liu et al., 1984) of the final report series.

Water Quality Analyses

Dissolved oxygen and pH were measured daily at all treatment levels for a period, usually a week, until we determined that the levels were stabilized. After this, pH and dissolved oxygen were determined at weekly intervals, alternating between the replicates. Dissolved oxygen was measured with a Yellow Springs Instrument Co. dissolved oxygen probe and pH with an Orion 407A Ionanalyzer. Temperature was monitored hourly in one of the control tanks using a Honeywell thermograph and checked weekly at all concentrations with a glass mercury thermometer. Hardness, alkalinity, and acidity were determined weekly in the diluent water using titration techniques (Hach Chemical Company, Sunnyvale, CA). Residual chlorine was also determined weekly in the diluent water using a Fischer and Porter

amperometric titrator (Arthur H. Thomas Co., Philadelphia, PA). The diluent water was also evaluated periodically for the presence of contaminants such as pesticides and PCBs. No detectable levels of these chemicals were found in any of the samples even though samples yielding suspicious peaks were further investigated by mass spectroscopy.

Statistical Analyses

General

Up to 31 variables were analyzed for each compound, including survival and growth measurements, fertility measures, and, in the case of fathead minnow chronic studies, various global or summary types of measures. Most of these variables were tested for each series separately and for the pooled series. Statistical analyses and graphics were produced on an IBM 3033 computer using the SAS statistical package and the VERSATEC plotter.

Transformations

In cases where homogeneity of variance assumptions were unwarranted, variance-stabilizing transformations were applied to variables before statistical tests or error-bar calculations were performed. These transformations were of two types (Bishop et al., 1975):

(1) Tukey arcsin transformation for proportions,

$$Y = \arcsin \sqrt{X/(N+1)}$$

where X/N is the proportion to be transformed;

(2) Square root transformation,

$$Y = \sqrt{X}$$

used for the F_0 fertility measures, which were assumed to be Poisson-distributed.

Unit of Analysis

The unit of analysis is the smallest experimental unit that yields a single observation. For example, both fry length and fry survival are measured for each fish (note that fry survival is a Bernoulli random variable valued 0 or 1 for each death or survival); whereas F_0 biomass, being a product of a chamber mean and a chamber proportion, yields only one value per chamber. Likewise, the total survivability and total productivity indices can only be measured on the aquarium level because they

include F_0 cumulative survival, which yields only one measurement per aquarium.

Series Included in the Analyses

In most cases, the data were analyzed for each series separately as well as for both series pooled. The exceptions were total survivability and total productivity indices, for which there were insufficient degrees of freedom to analyze the series separately.

Statistical Tests

The statistical tests were designed to detect statistically significant differences between control and treatment groups. It was assumed that any treatment effect would be detrimental to the organism, so that the tests were all one-tailed in the direction of greater mortality, smaller fry, or lower fertility. One of two types of tests was used, depending on the type of data analyzed:

(1) Proportional data, which included measures of egg and fry survival and fry deformity, were analyzed in an untransformed state using Fisher's Exact Test for analysis of 2 x 2 contingency tables. When the total sample size exceeded 40, the normal approximation to the hypergeometric distribution was used. Probability levels of less than 0.01 for each comparison (Miller, 1965) were flagged as statistically significant, yielding experiment-wise alpha levels of approximately 0.05.

(2) Nonproportional data were first subjected to an analysis of variance, using concentration and, where there were sufficient degrees of freedom, series and concentration-series interactions as independent variables. The mean square error from the ANOVA was then used to perform Dunnett's test of control-treatment differences (Dunnett, 1955).

Graphics

Two types of graphic displays were produced to aid in analyzing the data: detailed plots on the smallest level practical (chamber, breeding pair, or batch), and aquarium-level plots with error bars (where possible). For untransformed data, the error bar widths were the sample standard errors (e.g., the sample standard deviations divided by the square root of the sample size). In cases where the dependent variable underwent a variance-stabilizing transformation, the mean point for each series was plotted in its untransformed state, while the error bars represented the standard deviation of the mean point of the transformed data that were inversely transformed back into the original metric and bracketed around the untransformed point. The rationale for this approach was to provide a graphic illustration of the raw data while at the same time displaying

error bars that corresponded to the statistical tests that were performed on the transformed data. For example, survival data were transformed by the Tukey arcsin method. A set of theoretically correct error bars for the transformed data, based on underlying distribution assumptions, is given by the expression

$$\arcsin \sqrt{X/(N+1)} \pm 1/\sqrt{2N} ,$$

where X is the number of fry alive and N is the number that was transferred originally into an aquarium. When inversely transformed back into the original metric, the error bars become

$$\sin [\arcsin \sqrt{X/(N+1)} \pm 1/\sqrt{2N}]^2 .$$

Note that the error bars depend only on the sample size and the number alive pooled over chambers. Consequently, in a plot of aquaria survival proportions, an aquarium with widely differing survival proportions in its two chambers can have error bars similar to another aquarium where the two chambers have similar proportions.

Error bars were not plotted for the global indices of total survivability and total productivity, two variables that are composites of several other variables. The total survivability index was defined as the product of the cumulative F_0 survival to 180 days, the average number of eggs per female, and the cumulative F_1 survival after 60 days of exposure. The total productivity index was defined as the product of the total survivability index and the average weight of the F_1 generation after 60 days of exposure. Because an estimate of the variability for each aquarium would have required the questionable assumption that the component variables were all mutually independent, it was decided that error bars in this case might be misleading. Note that the statistical tests were performed using the mean squared error from a one-way ANOVA, allowing sufficient degrees of freedom for these variables.

Global Indices

In addition to the two global indices defined above, five additional such indices were used in analyzing the data from the fathead minnow chronic studies. These indices were defined as follows:

- F_0 90-day cumulative fry survival--fry survival at the end of 90 days as a proportion of the number of embryos exposed.
- F_0 178-day cumulative fry survival--fry survival at the end of the juvenile growth phase (up to the point where the sexes were identified and breeding pairs established) as a proportion of the number of embroys exposed.

- F_0 60-day biomass--the product of length at 60 days and 60-day fry survival.

- F_1 60-day cumulative fry survival--fry survival at the end of 60 days as a proportion of the number of embryos exposed.

- F_1 60-day standing crop--the product of weight at 60 days and 60-day fry survival.

RESULTS AND DISCUSSION

Early Life Stage Studies

Channel Catfish

As pointed out earlier, we encountered difficulties separating the eggs because they were past the initial hardening stage and were easily damaged when handled. Because the eggs were not separate, the fungus-affected eggs could not be removed to prevent the spread of disease to other eggs. Despite prophylactic treatment with malachite green up to the time of hatching, many of the eggs were lost to fungal infection. In addition, because we had to start the tests with clumps of eggs, the initial number of eggs varied markedly among the treatment groups. Nonetheless, data on egg hatchability and fry survival from tests performed on TNT and LAP water are shown in Tables 4 and 5, respectively.

Table 4. EFFECTS OF EXPOSURE TO TNT ON CHANNEL CATFISH EGGS AND FRY

Average Measured Concentration (mg/L)	Test Series	Eggs		Fry	
		Initial Number	% Hatch	Number Hatched	% Survival
Control	A	34	32.4	11	36.4
	B	38	26.3	10	40.0
0.11	A	40	50.0	20	55.0
	B	54	61.1	33	51.5
0.15	A	48	60.4	29	72.4
	B	43	32.5	14	42.8
0.30	A	59	61.0	36	58.3
	B	60	51.7	31	87.1
0.66	A	57	61.4	35	91.4
	B	51	64.7	33	84.8
1.35	A	67	55.2	37	62.2
	B	59	40.7	24	79.2

Table 5. EFFECTS OF 30 DAYS OF EXPOSURE TO LAP WATER ON CHANNEL CATFISH EGGS AND FRY

Average Measured Concentration (mg/L)	Test Series	Eggs		Fry	
		Initial Number	% Hatch	Number Hatched	% Survival
Control	A	41	56.1	23	43.5
	B	44	56.8	25	24.0
0.15	A	62	16.1	10	20.0
	B	41	14.6	6	50.0
0.36	A	21	28.6	6	33.3
	B	34	47.0	16	43.8
0.88	A	70	22.8	16	25.0
	B	57	50.9	29	41.4
1.96	A	70	42.8	30	6.7
	B	46	21.7	10	0.0
4.23	A	65	13.8	9	0.0
	B	33	0.0	0	0.0

Although only the grossest inferences can be made from these data, it appears that TNT concentrations as high as 1.35 mg/L had no effect on egg hatching success or fry survival. For LAP water, a concentration of 4.23 mg/L clearly reduced the percentage of eggs hatched, and a concentration of 1.96 mg/L reduced fry survival. For both of these tests, the eggs were exposed for approximately 10 days and the fry for the remainder of the exposure period.

Rainbow Trout

The effects of TNT and LAP on egg hatchability, fry survival, and fry growth are shown in Tables 6 and 7, respectively.

Table 6. EGG HATCHING SUCCESS, FRY SURVIVAL, AND FRY GROWTH IN RAINBOW TROUT EXPOSED TO TNT FOR 60 DAYS (Test 1)

Measured Concentration (mg/L)		Test	Eggs		Fry Alive	Average Fry	Average Fry
Mean	S.D.	Series	No. Exposed	No. Hatched	At 60 Days	Length (cm)	Weight (g)
0.00	—	A	61	61	32	2.43	0.108
		B	63	63	37	2.42	0.109
0.07	0.007	A	60	56	3[a]	2.44	0.173
		B	60	56	10[a]	2.27	0.110
0.12	0.050	A	60	59	30	2.33	0.100
		B	61	61	42	2.21[a]	0.093[a]
0.21	0.100	A	61	44[a]	12	2.54	0.163
		B	60	49[a]	21	2.45	0.130
0.49	0.200	A	60	59	26	2.21[a]	0.087[a]
		B	60	60	41	2.05[a]	0.080[a]
0.93	0.350	A	62	44[a]	14[a]	1.93[a]	0.067[a]
		B	64	37[a]	8[a]	1.96[a]	0.060[a]

[a]Statistically significant, $p < 0.05$.

Table 7. EGG HATCHING SUCCESS, FRY SURVIVAL AND FRY GROWTH IN RAINBOW TROUT EXPOSED TO LAP WATER FOR 60 DAYS

Measured Concentration (mg/L) Mean	S.D.	Test Series	Eggs No. Exposed	Eggs No. Hatched	Fry Alive At 60 Days	Average Fry Length (cm)	Average Fry Weight (g)
0.00	—	A	60	59	34	2.46	0.110
		B	60	59	21	2.55	0.130
0.06	0.009	A	60	60	36	2.34	0.100
		B	65	61	32	2.44	0.123
0.10	0.030	A	60	43	13	2.44	0.132
		B	60	50	11	2.62	0.178
0.21	0.040	A	60	43[a]	10	2.52	0.153
		B	61	35[a]	10	2.37	0.127
0.45	0.070	A	60	58	16	2.17[a]	0.113
		B	61	39	12	2.33[a]	0.131
0.92	0.180	A	60	56	31	2.02[a]	0.095[a]
		B	61	59	21	2.01[a]	0.093[a]

[a] Statistically significant, $p < 0.05$.

Egg hatching success was significantly reduced by exposure to TNT at concentrations of 0.21 and 0.93 mg/L. Because there was no effect on hatching success at 0.49 mg/L, the observed response at 0.21 mg/L was probably not toxicant-related. The number of fry that survived to the end of the experiment was reduced at the highest concentration (0.93 mg/L) compared with the controls. Survival was also reduced at a concentration of 0.07 mg/L; however, because no effect on survival was apparent at concentrations of 0.12 to 0.49 mg/L, we conclude that this effect was not toxicant-related.

Both length and weight were significantly reduced in both test series at concentrations of 0.49 and 0.93 mg/L and in Series B at 0.12 mg/L. The result at 0.12 mg/L was probably an artifact, because growth was not reduced in Series A at this concentration or in either series at the next higher concentration (0.21 mg/L). The effect on growth was appreciable; at 0.49 mg/L, length and weight were reduced approximately 12 and 23%, respectively, compared with the controls. At 0.93 mg/L, length and weight were similarly reduced by approximately 20 and by 41%, respectively.

LAP water did not appear to affect egg hatching success or fry survival within the range of concentrations tested (0.06 to 0.92 mg/L). A statistically significant effect on hatchability occurred in the pooled series at 0.21 mg/L, but because similar effects were not apparent at the two higher concentrations, we conclude that the observed response was not toxicant-related.

Statistically significant effects on length occurred at the two highest concentrations (0.45 and 0.92 mg/L) in both series. Weight was also reduced at a concentration of 0.92 mg/L. As with TNT, the effects on length and weight were appreciable; compared with the controls, length was reduced approximately 10 and 20% in the 0.45 and 0.92 mg/L concentrations, respectively, and weight was reduced 22% at 0.92 mg/L.

Unfortunately, because each treatment level in each of these tests represented the offspring of only one female, considerable variability existed in the observed responses, depending on the apparent susceptibility of the offspring from each female. As a result, the responses were generally similar for both series at a given treatment level but, except for the highest concentrations, did not appear to be concentration-related. An additional problem with these tests was the relatively low fry survival in the controls. In spite of very good hatching success, fry survival averaged only 56 and 47%, respectively, for the TNT and LAP controls.

Because of these problems, a second early life stage study with TNT was performed on rainbow trout using eggs that were randomized over the treatment levels. Data from this test are shown in Table 8.

TNT did not appear to affect egg hatching success at any of the test concentrations (0.02 to 1.69 mg/L). The number of deformed (e.g., spinal curvatures, two heads) fry present after hatching was monitored in this

Table 8. EGG HATCHING SUCCESS, FRY SURVIVAL AND FRY GROWTH IN RAINBOW TROUT EXPOSED TO TNT FOR 60 DAYS (Test 2)

Measured Concentration (mg/L)		Test	Eggs		No. Fry	Fry Alive	Average Fry	Average Fry
Mean	S.D.	Series	No. Exposed	No. Hatched	Deformed	At 60 Days	Length (cm)	Weight (g)
0.00	--	A	60	53	7	17[a]	4.25	0.750
		B	60	52	5	49	3.69	0.450
0.02	0.014	A	60	51	3	44	3.67[b]	0.490[b]
		B	60	55	9	44	3.61	0.425
0.04	0.018	A	60	55	8	42	3.68[b]	0.451[b]
		B	61	49	9	36	3.89	0.529
0.13	0.052	A	60	52	7	40	3.55[b]	0.430[b]
		B	60	50	2	44	3.57	0.411
0.24	0.076	A	60	52	7	36[b]	3.62[b]	0.435[b]
		B	61	51	6	37[b]	3.65	0.534
0.50	0.123	A	60	51	5	38[b]	3.25[b]	0.376[b]
		B	61	52	5	36[b]	3.37[b]	0.409
0.87	0.210	A	60	55	5	35[b]	3.11[b]	0.396[b]
		B	60	48	3	28[b]	3.03[b]	0.340[b]
1.69	0.328	A	62	56	6	3[b]	2.18[b]	0.155[b]
		B	60	56	6	2[b]	2.38[b]	0.180[b]

[a] 29 Fry were lost when tank flooded.
[b] Statistically significant, $p < 0.05$.

test and appeared to be unaffected by TNT. Sixty days after hatching, fry survival was reduced in both series compared with the controls at concentrations of 0.24 to 1.69 mg/L. Note that control survival at 60 days was adjusted in the statistical analysis for Series A to compensate for the loss of 29 fry that occurred when the tank overflowed several times between Days 28 and 32. This adjustment was made by extrapolating to the number of fry that would have survived based on the survival of the fry that did not wash out of the tank.

Exposure to TNT concentrations of 0.50 to 1.69 mg/L reduced the length of trout fry in both series compared with the controls. Length was also reduced at concentrations of 0.02 to 0.24 mg/L in Series A compared with controls, but this was most likely due to the reduced density of fry in the Series A controls, rather than to TNT. Fry in the control tank of this series were present at approximately one-third the density of fry in the exposure tanks and should have exhibited an increased growth rate. If the average length of fry in Series B is used as the norm, length was reduced approximately 10, 17, and 38% at concentrations of 0.50, 0.87, and 1.69 mg/L, respectively.

Trout fry weight exhibited a similar response, although the effect was significant only at the two highest concentrations (0.87 and 1.69 mg/L) in both series. Again using the average weight of fry in Series B controls as the norm, weight reductions of approximately 18 and 63% occurred at these two concentrations, respectively.

Water quality from the trout early life stage studies with LAP water and TNT are shown in Tables 9 through 11. Temperature was monitored continuously at hourly intervals. Based on 50 random monitorings, the average temperature for the test with LAP water and the first test with TNT was 12.7°C, with a standard deviation of 0.57 and a range of 11.8-13.8°C. For the second test with TNT, the average temperature was 11.3°C, with a standard deviation and range of 1.01 and 10.0-13.0°C, respectively.

Table 9. WATER QUALITY PARAMETERS MONITORED DURING TROUT EARLY LIFE STAGE STUDY WITH LAP WATER

Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Conductivity (μmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n
Control	10.3	0.5	9.6-10.8	4	7.5	0.4	7.0-7.9	9	130	94	60-280	9	71	43	24-110	5	52	36	20-100	8
0.06	10.0	0.6	9.6-10.4	2	7.4	0.4	7.0-8.0	4	110	86	62-240	4	46	38	24-90	3	43	32	20-80	3
0.10	9.9	0.7	9.4-10.4	2	7.4	0.5	7.0-8.1	4	113	86	61-242	4	50	43	25-100	3	47	38	20-90	3
0.21	9.9	0.7	9.4-10.4	2	7.4	0.5	7.0-8.1	4	114	88	60-245	4	50	43	25-100	3	43	32	20-80	3
0.45	10.0	0.8	9.4-10.6	2	7.5	0.5	7.0-8.1	4	113	86	60-242	4	53	49	24-110	3	43	32	20-80	3
0.92	9.8	0.6	9.4-10.3	2	7.5	0.5	7.0-8.1	4	107	82	60-230	4	46	38	24-90	3	43	32	20-80	3

Table 10. WATER QUALITY PARAMETERS MONITORED DURING TROUT EARLY LIFE STAGE STUDY WITH TNT (Test 1)

Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Conductivity (μmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n
Control	10.2	0.6	9.4-10.8	4	7.5	0.4	7.0-8.0	9	132	91	62-270	9	66	45	24-120	6	49	34	20-100	8
0.07	10.0	0.8	9.4-10.5	2	7.4	0.4	7.0-8.0	4	108	75	62-220	4	48	40	24-95	3	47	38	20-90	3
0.12	9.9	0.7	9.4-10.4	2	7.5	0.4	7.0-8.0	4	114	76	61-230	4	50	44	24-100	3	43	32	20-80	3
0.21	9.9	0.7	9.4-10.4	2	7.5	0.4	7.0-8.0	4	110	80	62-230	4	50	43	24-100	3	47	38	20-90	3
0.49	10.0	0.8	9.4-10.5	2	7.5	0.4	7.0-8.0	4	110	80	61-230	4	51	43	24-100	3	47	38	20-90	3
0.93	9.9	0.7	9.4-10.4	2	7.5	0.4	7.0-8.0	4	110	77	61-225	4	50	43	24-100	3	47	38	20-90	3

Table 11. WATER QUALITY PARAMETERS MONITORED DURING TROUT EARLY LIFE STAGE STUDY WITH TNT (Test 2)

Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Conductivity (μmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n
Control	11.2	1.4	10.0-13.4	6	7.3	0.3	6.9-7.8	11	140	39	70-205	11	51	6	40-58	11	47	5	38-56	11
0.02	10.8	1.8	8.5-13.0	6	7.4	0.2	7.2-7.6	4	80	14	70-90	2	41	1.4	40-42	2	38	3	36-40	2
0.04	10.4	1.9	8.2-12.8	6	7.3	0.2	7.1-7.6	4	78	18	65-90	2	43	4	40-46	2	38	3	36-40	2
0.13	10.4	1.8	8.4-12.4	6	7.3	0.2	7.1-7.5	4	78	18	65-90	2	46	3	44-48	2	40	6	36-44	2
0.24	10.2	1.6	8.4-12.2	6	7.4	0.1	7.2-7.5	4	92	4	90-95	2	44	6	40-48	2	41	4	38-44	2
0.50	10.2	1.7	8.4-12.6	6	7.4	0.1	7.3-7.5	4	88	6	85-90	2	42	6	38-46	2	39	7	34-44	2
0.87	10.3	1.4	8.6-12.0	6	7.4	0.1	7.4-7.5	4	82	4	80-85	2	43	7	38-48	2	38	6	34-42	2
1.69	10.7	1.0	10.0-12.2	6	7.6	0.2	7.4-7.9	4	88	4	85-90	2	42	3	40-44	2	39	4	36-42	2

Fathead Minnows

The results of the 30-day early life stage study with TNT are shown in Table 12.

Table 12. EGG HATCHING SUCCESS AND FRY SURVIVAL IN FATHEAD MINNOWS EXPOSED TO TNT FOR 30 DAYS

Measured Concentration (mg/L)		Test	Eggs		Fry Alive	Average Fry
Mean	S.D.	Series	No. Exposed	No. Hatched	At 30 Days	Length (cm)
0.00	--	A	60	60	32	1.03
		B	60	57	27	1.05
0.07	0.017	A	60	57	36	1.08
		B	60	57	35	1.08
0.10	0.022	A	60	60	36	1.12
		B	60	59	36	1.14
0.16	0.079	A	60	53	40	1.03
		B	60	58	37	1.08
0.42	0.081	A	60	41	26	1.12
		B	60	54	25	1.10
0.84	0.178	A	60	55	15	1.14
		B	60	57	21	0.93

Because this test was designed strictly as a range-finding study, no statistical analyses were performed on the data. However, it appears that egg hatching success was not reduced within a concentration range of 0.07 to 0.84 mg/L. Fry survival was reduced at 0.84 mg/L after 30 days of exposure in both series, and growth was reduced in Series B at this concentration.

The results of the fathead minnow early life stage study on LAP water are summarized in Table 13. Although the data from this range-finding test were not statistically analyzed, the results indicate that egg survival and fry growth were not impaired within the range of concentrations tested (0.05 to 2.68 mg/L). However, marked effects on fry survival occurred at 1.02 and 2.68 mg/L.

Both of these tests were characterized by relatively poor fry survival (~ 50%) in contrast to the very good hatching success. In spite of this, the marked reduction in fry survival at the higher treatment levels strongly suggests toxicant-related responses.

Table 13. EGG HATCHING SUCCESS AND FRY SURVIVAL IN FATHEAD MINNOWS EXPOSED TO LAP WATER FOR 30 DAYS

Measured Concentration (mg/L) Mean	S.D.	Test Series	No. Exposed	No. of Eggs Hatched (n=60)	Fry Alive At 30 Days	Average Fry Length (cm)
Control	—	A	60	60	37	1.05
		B	60	52	29	1.03
0.05	0.007	A	60	57	50	0.91
		B	60	56	43	0.94
0.53	0.057	A	60	58	26	1.01
		B	60	59	33	0.99
1.02	0.173	A	60	60	23	0.99
		B	60	60	23	1.01
2.68	0.389	A	60	60	4	0.93
		B	60	59	2	0.95

Water quality associated with these tests are summarized in Tables 14 and 15.

Table 14. WATER QUALITY PARAMETERS MONITORED DURING FATHEAD MINNOW EARLY LIFE STAGE STUDY WITH TNT

Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Conductivity (μmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)				Acidity (mg/L $CaCO_3$)			
	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n
Control	8.4	0.22	8.1-8.8	8	7.8	0.41	7.4-8.2	4	28	13	10-40	4	15	10	8-30	4	10	0		4	10	0		4
0.07	8.3	0.20	8.0-8.5	8																				
0.10	8.4	0.17	8.1-8.6	8																				
0.16	8.3	0.13	8.2-8.5	8																				
0.42	8.3	0.17	8.1-8.5	8																				
0.84	8.3	0.24	8.0-8.7	8	8.0	0.38	7.7-8.4	4	28	12	10-35	4	18	12	10-35	4	10	0		4	10	0		4

Table 15. WATER QUALITY PARAMETERS MONITORED DURING FATHEAD MINNOW EARLY LIFE STAGE STUDY WITH LAP WATER

Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Conductivity (μmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)				Acidity (mg/L $CaCO_3$)			
	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n
Control	7.6	0.51	6.9-8.5	12	7.4	0.20	7.2-7.7	6	112	39	73-170	6	35	10	25-49	6	39	14	20-55	6	6	1	5-8	6
0.05	7.5	0.45	7.0-8.5	12		--				--				--				--				--		
0.53	7.5	0.52	6.8-8.5	12		—				--				--				—				--		
1.02	7.5	0.37	6.9-8.3	12		—				--				--				--				--		
2.68	7.6	0.34	7.1-8.4	12	7.3	0.15	7.1-7.5	6	101	24	72-138	6	40	6	32-50	6	33	6	25-40	6	26	9	15-40	6

Chronic Studies

Fathead Minnows

TNT. Data on egg hatching success, fry survival and deformities and fry growth are shown in Table 16.

The number of eggs that survived to hatch was reduced in Series B at a concentration of 1.21 mg/L compared with the controls. Fry survival was reduced in Series A at concentrations of 0.25 to 1.21 mg/L for exposure periods of 30 to 178 days. Fry survival was reduced in Series B only at 1.21 mg/L for all exposure periods, except that a statistically significant effect was apparent at 0.56 mg/L after 178 days of exposure.

Fry length was reduced in Series A at 0.56 and 1.21 mg/L after 30 days of exposure. This response was apparently ameliorated over time, because it was barely discernible after 60 days of exposure and not at all apparent after 90 days.

Table 16 EGG HATCHING SUCCESS, FRY SURVIVAL, AND FRY GROWTH IN FATHEAD MINNOWS AFTER CHRONIC EXPOSURE TO TNT

Average Actual Concentration (mg/L)	Test Series	Eggs		No. Fry Deformed	Fry Survival						Fry Growth (cm)		
					Days Exposed						Days Exposed		
		No. Exposed	No. Hatched		30	60	90	120	150	178	30	60	90
Control	A	100	96	3	87	86	81	81	80	80	1.88	2.57	3.15
	B	110	90	2	74	73	72	72	72	71	1.80	2.98	3.90
0.04	A	100	100	3	89	89	83	79	77	77	1.89	2.54	3.14
	B	110	97	0	72	71	69	69	69	67	1.99	2.92	3.67
0.10	A	100	96	1	89	85	84	81	80	78	1.93	2.49	3.10
	B	110	78	1	54	54	53	53	55	55	2.01	3.14	4.09
0.25	A	100	99	2	77[a]	76[a]	73[b]	69[a]	67[a]	64[a]	1.86	2.63	3.11
	B	110	89	0	61[b]	61[b]	59[b]	59[b]	59[b]	59[b]	2.00	3.01	4.20
0.56	A	100	96	4	73[a]	68[a]	65[a]	55[a]	52[a]	48[a]	1.59[a]	2.40	3.12
	B	110	82	1	57[b]	56[b]	55[b]	54[b]	53[b]	47[a]	1.94	3.10	3.85
1.21	A	100	99[b]	5	43[a]	40[a]	33[a]	25[a]	22[a]	22[a]	1.70[a]	2.61	3.22
	B	110	68[a]	0	18[a]	15[a]	13[a]	10[a]	9[a]	7[a]	2.05	3.51	4.58

[a] Statistically significant, $p < 0.05$.

[b] Statistically significant, $p < 0.05$, if the series are pooled.

The effect of TNT on reproduction is shown in Table 17. These data indicate that most, if not all, of the reproductive parameters were adversely affected by TNT within a concentration range of 0.04 to 1.21 mg/L. Even in instances where the affected parameters were not statistically different from the controls, they were generally lower than the control values. The number of eggs per spawn was the parameter least affected by TNT, indicating that the overall effect on reproduction was due to reductions in the survival of the breeding pairs and frequency of spawns per pair.

Table 17. REPRODUCTIVE PARAMETERS IN FATHEAD MINNOWS AFTER CHRONIC EXPOSURE TO TNT

Average Actual Concentration per (mg/L) Pair/Day	Test Series	Spawning Pair Survival (days)	No. of Spawns/ Pair	Eggs per Pair	Eggs per Spawn	Eggs
Control	A	113	16	2948	184.2	26.1
	B	201	29	5054	174.3	25.1
0.04	A	82[b]	12	1954[b]	162.8	23.8
	B	56[a]	8[a]	1255[b]	156.9	22.4
0.10	A	104[b]	5[a]	898[a]	179.6	8.6[b]
	B	81[a]	5[a]	444[a]	88.8	5.5[b]
0.25	A	84	7[b]	1450[b]	207.1	17.3
	B	145	12[b]	1839[b]	153.2	12.7
0.56	A	77[b]	3[a]	209[a]	69.7	2.7[a]
	B	85[a]	1[a]	120[a]	120.0	1.4[a]
1.21	A	81[b]	0[a]	0[a]	0[a]	0[a]
	B	43[a]	0[a]	0[a]	0[a]	0[a]

[a]Statistically significant, $p < 0.05$.
[b]Statistically significant, $p < 0.05$, if the series are pooled.

The effect of TNT on the F_1 generation is shown in Table 18. A statistically significant reduction in the number of eggs hatched occurred

Table 18. EFFECT OF CHRONIC EXPOSURE TO TNT ON F_1 FATHEAD MINNOWS

Average Actual Concentration (mg/L)	Test Series	Eggs			Fry at 30 Days				Fry at 60 Days			
		No. Exposed	No. Hatched	No. Fry Deformed	No. Transferred	No. Survived	Length (cm)	Weight (g)	No. Transferred	No. Survived	Length (cm)	Weight (g)
0	A	1150	777	26	79	71	1.91	0.050	91	59	2.87	0.174
	B	2000	1432	53	78	76	1.82	0.050	73	61	2.72	0.182
0.04	A	850	583[b]	25	77	52[a]	1.88	0.076	82	66	2.58[a]	0.121[a]
	B	950	388[a]	19	38	30[a]	1.88	0.050	34	28	2.90[b]	0.227[b]
0.10	A	450	287[b]	39[a]	36	34[b]	1.70[a]	0.041	45	37	2.46[a]	0.097[a]
	B	345	183[a]	6[b]	31	12[a]	1.81[b]	0.056	38	34	2.68[b]	0.147[a]
0.25	A	750	497[b]	82[a]	62	60[b]	1.93	0.064	71	58	2.73[a]	0.161[b]
	B	848	490[a]	57[a]	73	33[a]	2.01	0.081	66	60	2.66[b]	0.162[b]
0.56	A	250	127[b]	14[a]	0	—	—	—	44	44	2.29[a]	0.084[a]
	B	150	52[a]	2[b]	0	—	--	--	0	--	--	--
1.21	A	0	0	0	0	—	--	-	0	—	—	--
	B	0	0	0	0	—	—	--	0	—	--	--

[a] Statistically significant, $p < 0.05$.

[b] Statistically significant, $p < 0.05$ if series are pooled.

in Series B at concentrations of 0.04 to 0.56 mg/L. Although no statistically significant effect occurred in Series A at any of these concentrations, all values were less than the control and the overall effect was significant when the series were pooled.

The number of deformed fry present after hatching was significantly increased compared with the controls in Series A and the pooled series at concentrations of 0.10 to 0.56 mg/L. The effect was also significant in Series B at 0.25 mg/L.

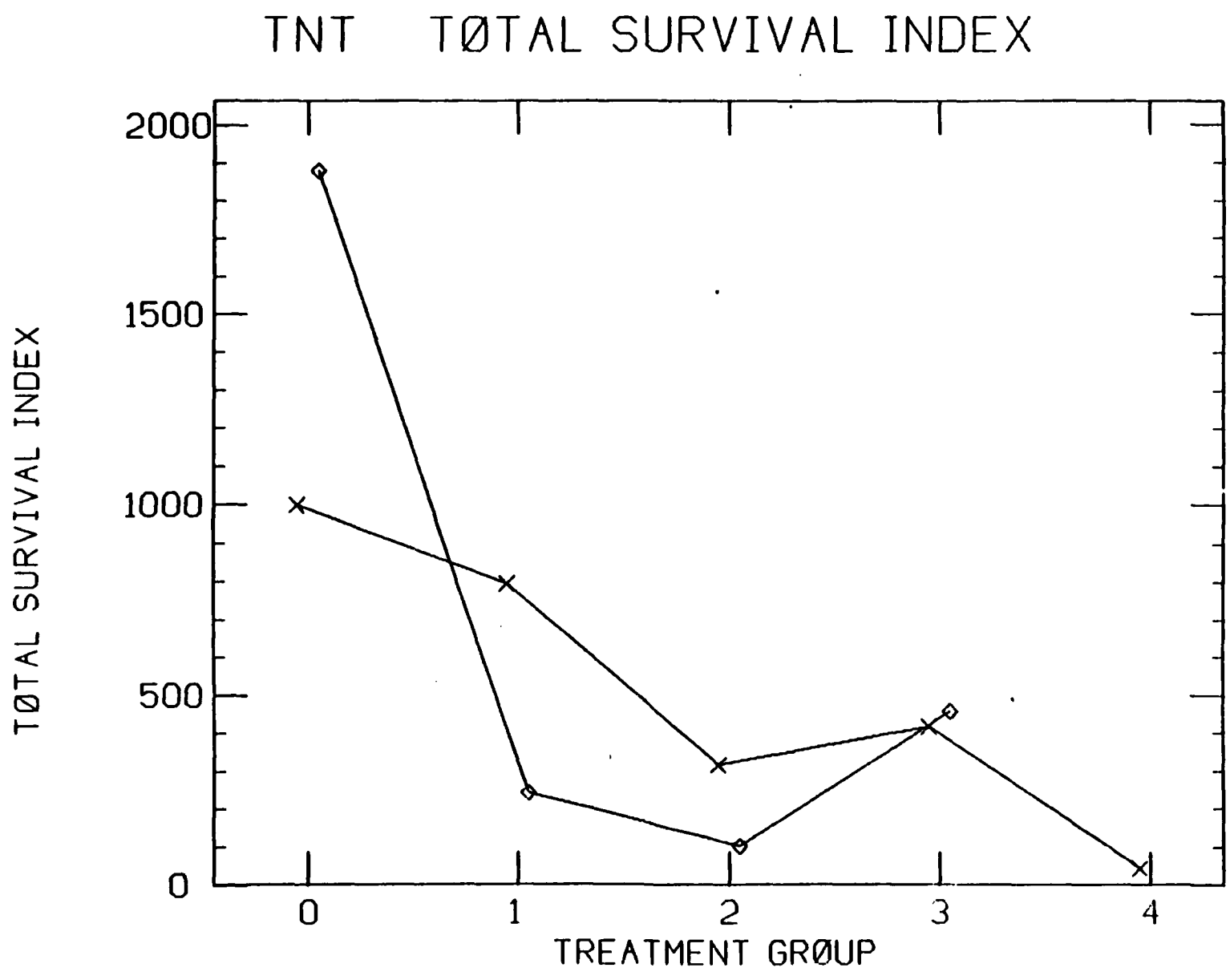
Survival of F_1 fry after 30 days of exposure to TNT was adversely affected in Series B and in the pooled series at concentrations of 0.04 to 0.25 mg/L. A significant effect on survival also occurred in Series A at 0.04 mg/L, but this may have been an artifact because no such effect was apparent in Series A at 0.10 and 0.25 mg/L. There was no apparent effect on the survival of F_1 fry reared to 60 days.

The effect of TNT on the growth of F_1 fry was primarily apparent in the 60-day exposure groups. Length was significantly reduced in Series A and in the pooled series at concentrations ranging between 0.04 to 0.56 mg/L. Weight was also significantly reduced at these concentrations in the pooled series, at concentrations of 0.04, 0.10, and 0.56 mg/L in Series A alone, and at 0.10 mg/L in Series B alone. At the lowest concentration tested (0.04 mg/L), the reductions in length and weight were approximately 2% in the pooled series; in Series A alone, however, length was reduced by about 10% and weight by about 30%.

The effects of TNT on the global indices of total survivability and total productivity are shown in Figures 1 and 2 and summarized in Table 19. Both of these indices indicate that TNT had a deleterious effect on fathead minnows at all of the concentrations tested (0.04 to 1.21 mg/L).

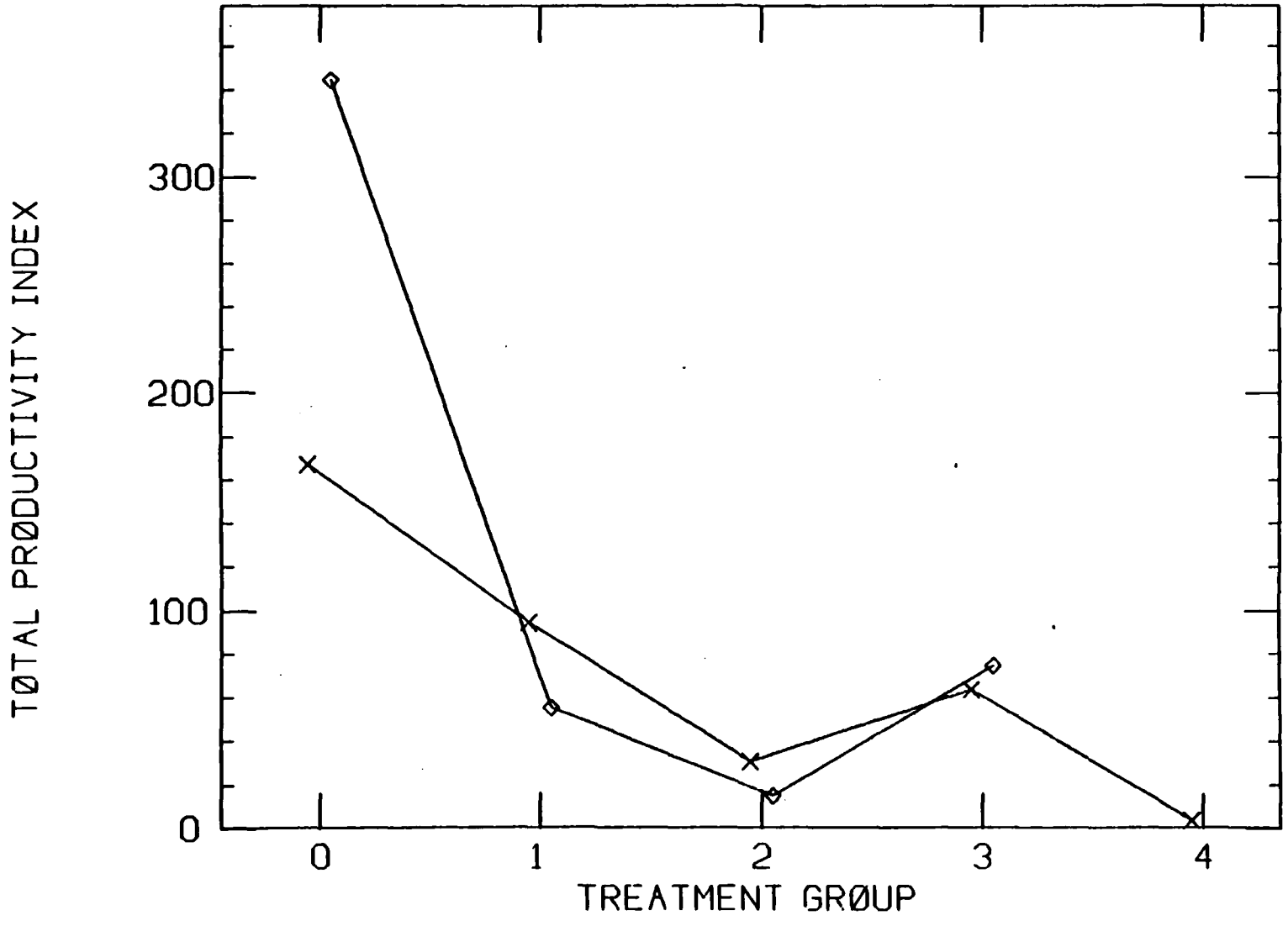
Table 19. TOTAL SURVIVABILITY AND PRODUCTIVITY INDICES IN FATHEAD MINNOWS AFTER CHRONIC EXPOSURE TO TNT

Average Actual Concentration (mg/L)	Total Survivability		Total Productivity	
	Series A	Series B	Series A	Series B
Control	999	1880	167	345
0.04	795	244	95	55
0.10	318	102	31	15
0.25	419	458	64	75
0.56	45	0	4	0
1.21	0	0	0	0



Legend: 0 = Control, 1 = 0.04, 2 = 0.10, 3 = 0.25, 4 = 0.56, 5 = 1.21 mg/L TNT
X = Test Series A, ◊ = Test Series B

TNT TØTAL PRØDUCTIVITY INDEX



Legend: 0 = Control, 1 = 0.04, 2 = 0.10, 3 = 0.25, 4 = 0.56, 5 = 1.21 mg/L TNT
X = Test Series A, ◇ = Test Series B

The results of chemical analyses associated with this test are summarized in Table 20.

Table 20. TNT CONCENTRATIONS OBTAINED DURING THE FATHEAD MINNOW CHRONIC STUDY[a]

Nominal Concentration (mg/L)	Analyzed Concentration (mg/L)			
	Mean	S.D.	Range	n
Control	--	--	--	81
0.06	0.04	0.01	0.00-0.08	77
0.12	0.10	0.02	0.04-0.16	78
0.25	0.25	0.05	0.10-0.37	79
0.50	0.56	0.08	0.37-0.82	78
1.00	1.21	0.17	0.79-1.50	90

[a] Samples corresponding to nine observed diluter malfunctions were not included in averages.

LAP water. Data on egg hatching success, fry survival and deformities, and fry growth are shown in Table 21. There was no effect on egg hatching success within a concentration range of 0.05 to 1.36 mg/L. A significant proportion of fry that hatched at 1.36 mg/L was deformed (primarily scoliosis) in Series B and in the pooled series. Fry survival was consistently reduced in both series at 0.62 and 1.36 mg/L throughout this phase of the study. A statistically significant reduction in fry survival also occurred in the pooled series at 0.28 mg/L after 30 days of exposure but was not apparent at later exposure periods.

Length of fry did not appear to be reduced in Series A at any of the concentrations tested during exposure periods of 30 to 60 days. In Series B, significant effects on growth occurred at concentrations of 0.11 to 1.36 mg/L after 30 days of exposure. This effect was significant only at 0.11 mg/L after 60 days of exposure and was significant in none of the concentrations after 90 days of exposure.

The effect of LAP water on reproduction is shown in Table 22. The results of the statistical analyses indicate that reproduction was significantly affected only at 1.36 mg/L. However, it should be noted that in Series A, the number of eggs per pair, spawns per pair, eggs per spawn, and eggs per pair/day, were reduced at all concentrations (0.05 to 1.36 mg/L) compared with the controls.

Table 21. EGG HATCHING SUCCESS, FRY SURVIVAL, AND FRY GROWTH IN FATHEAD MINNOWS AFTER CHRONIC EXPOSURE TO LAP WATER

Average Measured Concentration (mg/L)	Test Series	Eggs		No. Fry Deformed	Fry Survival						Fry Growth (cm)		
					Days Exposed						Days Exposed		
		No. Exposed	No. Hatched		30	60	90	120	150	181	30	60	90
Control	A	120	93	1	78	77	77	77	76	75	1.91	2.99	3.69
	B	120	96	1	81	78	78	78	78	74	2.00	2.97	3.74
0.05	A	120	91	0	75	75	75	74	74	72	1.98	3.08	3.73
	B	120	106	2	84	84	84	84	84	82	1.95	2.89	3.54
0.11	A	120	88	2	64	64	64	64	64	65	2.07	3.11	3.76
	B	120	103	0	79	76	76	76	75	72	1.57[a]	2.74[a]	3.52
0.28	A	120	90	4	71	71	71	71	71	70	1.92	2.92	3.61
	B	120	107	4	70	70	70	70	70	68	1.85[a]	2.88	3.61
0.62	A	120	90	3	50[a]	50[a]	50[a]	49[a]	49[a]	46[a]	1.92	3.22	3.85
	B	120	107	6	65[a]	65[a]	65[a]	64[a]	64[a]	64	1.80[a]	2.84	3.58
1.36	A	120	80	5	20[a]	18[a]	17[a]	17[a]	16[a]	16[a]	1.86	3.32	4.14
	B	120	98	10[a]	52[a]	49[a]	49[a]	46[a]	43[a]	36[a]	1.59[a]	2.82	3.57

[a] Statistically significant; $p < 0.05$.

Table 22. REPRODUCTIVE PARAMETERS IN FATHEAD MINNOWS AFTER CHRONIC EXPOSURE TO LAP WATER

Average Measure Concentration (mg/L)	Test Series	Spawning Pair Survival (days)	No. of Spawns Pair	Eggs/Pair	Eggs/Spawn	Eggs per Pair/Day
Control	A	77	12	2632	219.3	34.2
	B	93	11	2146	195.1	23.1
0.05	A	95	10	1634	163.4	17.2
	B	70	16	2331	145.7	33.3
0.11	A	86	12	1927	160.6	22.4
	B	122	18	3525	195.8	28.9
0.28	A	95	12	2054	171.2	21.6
	B	106	15	2556	170.4	24.1
0.62	A	104	7	1146	163.7	11.0
	B	120	14	2289	163.5	19.1
1.36	A	120	1[a]	27[a]	27[a]	0.2[a]
	B	78	1[a]	5[a]	5[a]	0.1[a]

[a] Statistically significant; $p < 0.05$.

The effects of LAP on the F_1 generation are shown in Table 23. Egg hatching success was reduced in Series A at 0.05, 0.62, and 1.36 mg/L. However, the response at 0.05 mg/L does not appear to be dose-related in view of the comparative lack of effect on this parameter at concentrations of 0.11 and 0.28 mg/L. A similar, apparently non-dose-related effect occurred in Series B at 0.11 mg/L, but no effect occurred at the higher concentrations of 0.28 and 0.62 mg/L. The proportion of deformed fry present after hatching was significantly increased in Series A at concentrations of 0.05, 0.28, and 1.36 mg/L. The effects at 0.05 and 0.28 mg/L were probably not toxicant-related in view of the lack of any apparent dose response at the other intermediate concentrations. There was no increase in the proportion of deformed fry in Series B at any of the exposure concentrations (0.05 to 0.62 mg/L).

No statistically significant effects on survival were found in F_1 fry reared to 30 days. Significant effects on survival were found for fry reared to 60 days, but the results were highly variable. Effects were found in Series A at 0.28 mg/L and in Series B at 0.05, 0.11, and 0.62 mg/L. The reductions in survival were significant in the pooled series at 0.28 to 1.36 mg/L. Based on the nonsignificant response in Series B at 0.28 mg/L, it is possible that the reduced survival that occurred at 0.05 and 0.11 mg/L was not toxicant-related. If the egg and fry survival for those fry that were reared to 60 days are pooled, the effect on the cumulative fry survival is limited to concentrations of 0.28 to 1.36 mg/L in Series A and in the pooled series. Cumulative fry survival was not significantly affected in Series B at concentrations of 0.05 to 0.62 mg/L.

Growth parameters for F_1 fry were also quite variable. Length was significantly reduced in Series A at 0.11 and 0.28 mg/L and in Series B at 0.11 mg/L after 30 days of exposure. Based on the length of fry exposed to concentrations of 0.28 and 0.62 mg/L in Series B, it appears likely that the response observed in Series B at 0.11 mg/L was not toxicant-related. The reductions in length observed in Series A at 0.11 and 0.28 mg/L correspond to 6.5% and 4.9%, respectively, of the control values. Although significant reductions in weight occurred at 0.11 mg/L in Series A and at 0.05 and 0.11 mg/L in Series B, the lack of any effect on weight at concentrations of 0.28 and 0.62 mg/L suggests that the effects at the lower concentrations were not toxicant-related.

Length and weight were reduced in Series A fry reared for 60 days in a LAP water concentration of 0.62 mg/L. This effect was probably toxicant-related in spite of the lack of response at the next higher concentration (1.36 mg/L) because the higher values at 1.36 mg/L probably reflected the reduced density of fish present in the larval rearing chambers.

Table 23. EFFECT OF CHRONIC EXPOSURE TO LAP WATER ON F_1 FATHEAD MINNOWS

Average Measured Concentration (mg/L)	Test Series	Eggs			Fry at 30 Days				Fry at 60 Days			
		No. Exposed	No. Hatched	No. Fry Deformed	No. Transferred	No. Survived	Length (cm)	Weight (g)	No. Transferred	No. Survived	Length (cm)	Weight (g)
Control	A	950	792	43	84	54	1.84	0.058	83	60	2.62	0.144
	B	743	595	75	69	60	1.91	0.065	78	76	2.35	0.106
0.05	A	650	506[a]	47[a]	68	49	2.08	0.079	75	56	2.52	0.142
	B	1001	781	45	85	79	1.85	0.056[a]	90	77[a]	2.34	0.107
0.11	A	800	631	37	88	70	1.72[a]	0.045[a]	64	56	2.61	0.150
	B	1300	865	72	89	73	1.66[a]	0.036[a]	70	48[a]	2.76	0.161
0.28	A	800	689	88[a]	79	50	1.75[a]	0.061	81	32[a]	2.98	0.266
	B	1045	843	42	76	57	1.83	0.058	85	81	2.38	0.098
0.62	A	550	389[a]	28	44	19	--	--	63	39	2.04[a]	0.080[a]
	B	950	759	26	74	60	1.86	0.068	81	61[a]	2.30	0.106
1.36	A	92	43[a]	10[a]	0	--	--	--	21	11[a]	2.84	0.280
	B	0	--	--	--	--	--	--	--	--	--	--

[a] Statistically significant; $p < 0.05$.

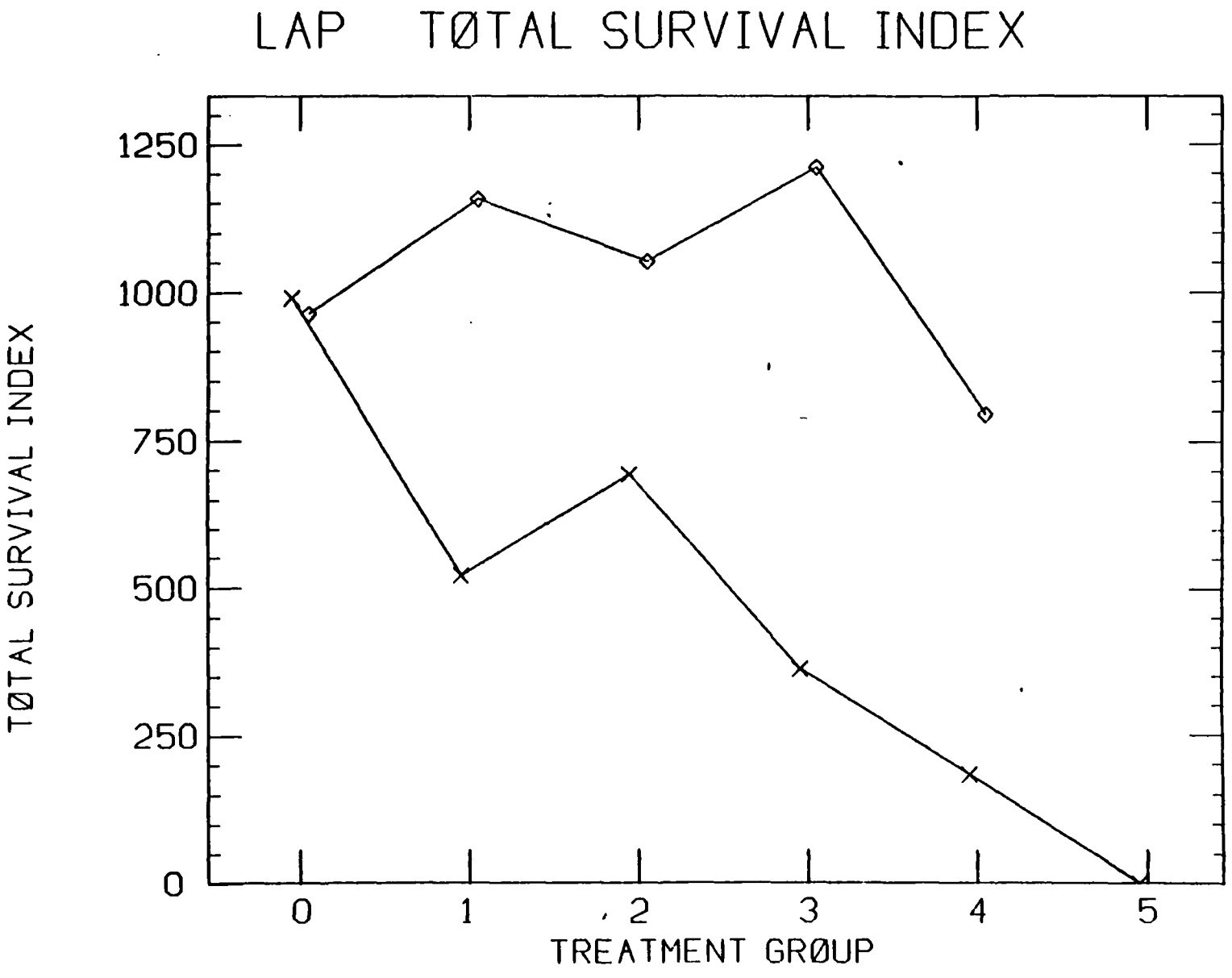
The effect of LAP water on total survivability and productivity indices is shown in Table 24 and in Figures 3 and 4. These parameters indicate that there was very little effect in Series B except at the two highest concentrations--0.62 and 1.36 mg/L. However, there appears to be a dose-related reduction in these indices in Series A at all of the test concentrations (0.05 to 1.36 mg/L).

Table 24. TOTAL SURVIVABILITY AND PRODUCTIVITY INDICES IN FATHEAD MINNOWS AFTER CHRONIC EXPOSURE TO LAP WATER

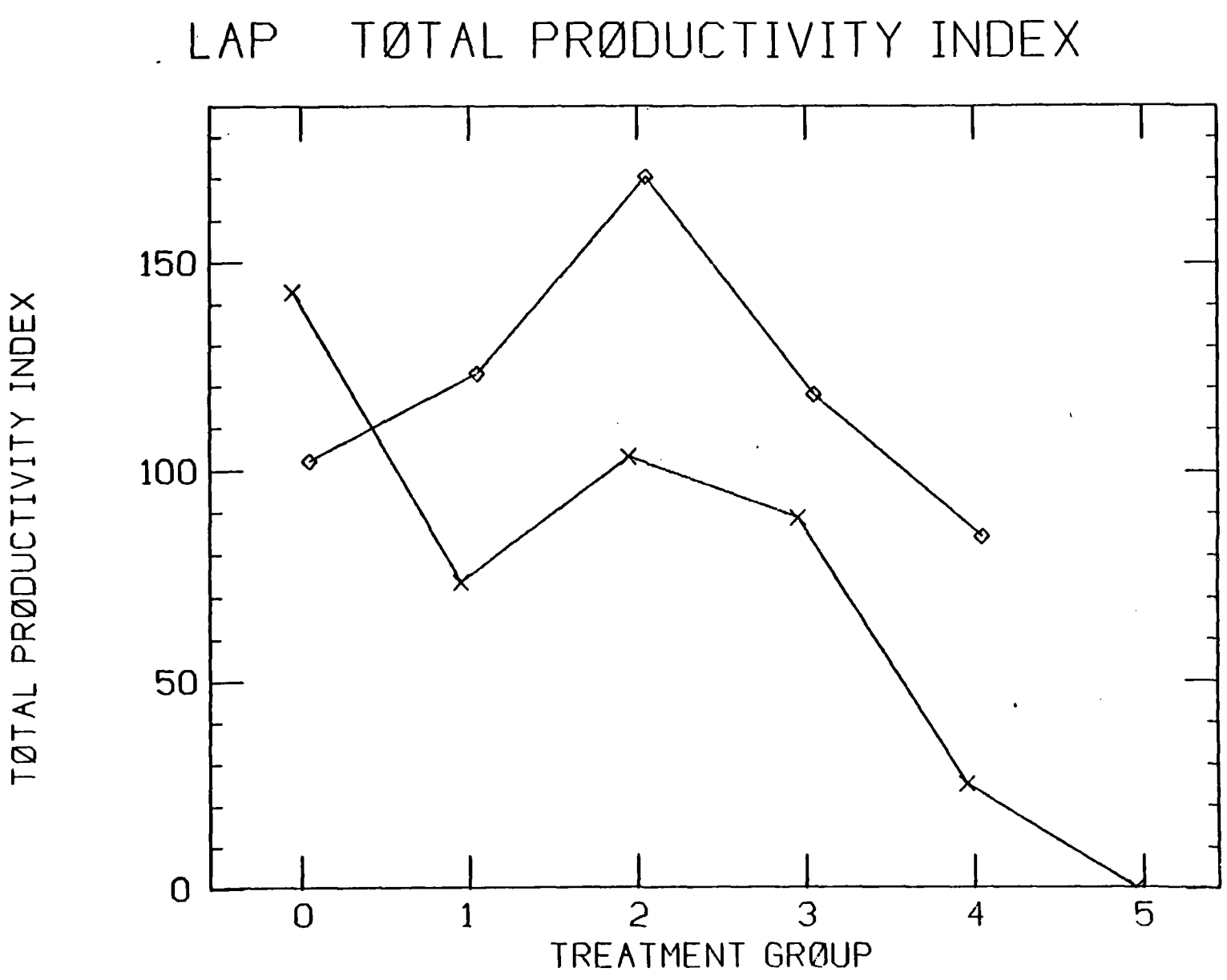
Average Actual Concentration (mg/L)	Total Survivability		Total Productivity	
	Series A	Series B	Series A	Series B
Control	992	963	143	102
0.05	523	1158	74	123
0.11	694	1013	103	164
0.28	364	1211	89	118
0.62	185	796	25	84
1.36	1	0	0.2	0

Chemical concentrations associated with the chronic study on LAP water are summarized in Table 25.

Water quality parameters associated with the chronic studies on TNT and LAP water are summarized in Tables 26 and 27.



Legend: 0 = Control, 1 = 0.05, 2 = 0.11, 3 = 0.28, 4 = 0.62, 5 = 1.36 mg/L TNT
X = Test Series A, ◊ = Test Series B



Legend: 0 = Control, 1 = 0.05, 2 = 0.11, 3 = 0.28, 4 = 0.62, 5 = 1.36 mg/L TNT
X - Test Series A, ◇ = Test Series B

Table 25. CHEMICAL CONCENTRATIONS OBTAINED DURING THE FATHEAD MINNOW CHRONIC STUDY ON LAP WATER[a]

Concentration (mg/L)	Analyzed Concentration (mg/L)			
	Mean	S.D.	Range	n
Control	0.00	0.00	—	54
0.06	0.05	0.02	<0.02–0.18	54
0.12	0.11	0.04	<0.05–0.29	54
0.25	0.28	0.08	0.14–0.46	54
0.50	0.62	0.14	0.35–0.98	54
1.00	1.36	0.28	0.85–2.21	68

[a]Samples corresponding to five observed diluter malformations were not included in the averages.

Table 26. WATER QUALITY PARAMETERS MONITORED DURING A FATHEAD MINNOW CHRONIC STUDY WITH TNT

Concentration (mg/L)	Dissolved oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	Mean	SD	Range	n	Mean	SD	Range	n	Mean	SD	Range	n	Mean	SD	Range	n	Mean	SD	Range	n	Mean	SD	Range	n
Control	6.9	0.7	5.2-8.5	55	7.6	0.3	6.9-8.5	48	24.5	0.8	23-27	28	123	58	38-295	48	39	18	13-80	44	36	18	12-80	49
0.06	6.8	0.8	4.5-8.6	55	7.5	0.4	6.8-8.7	48	24.6	0.8	23-27	25	122	55	40-272	48								
0.12	6.9	0.7	4.9-8.4	55	7.5	0.3	6.7-8.4	48	24.6	0.8	23-27	24	124	58	40-280	48								
0.25	6.8	0.9	4.3-8.5	55	7.5	0.4	6.9-8.6	48	24.6	0.8	23-27	23	124	58	35-290	48								
0.50	6.9	0.7	5.2-8.8	55	7.6	0.4	6.9-8.8	48	24.4	0.8	23-27	24	122	58	35-278	48								
1.00	7.6	0.5	6.1-8.5	55	7.7	0.3	7.1-8.5	48	24.7	0.9	23-27	21	122	55	35-285	48								

Table 27. WATER QUALITY PARAMETERS MONITORED DURING A FATHEAD MINNOW CHRONIC STUDY ON LAP WATER

Concentration (mg/L)	Dissolved oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)				
	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n
Control	6.6	0.8	5.0-8.4	50	7.5	0.4	6.9-8.6	51	25.6	0.6	24.5-26.8	17	94	64	28-298	49	33	27	9-129	45	31	21	10-100	47
0.05	6.4	0.9	4.6-8.4	50	7.5	0.4	6.9-8.7	51	25.2	0.4	24.5-26.0	16	96	64	28-300	49		--				--		
0.11	6.6	0.8	5.0-8.4	50	7.5	0.4	6.8-8.7	51	25.2	0.5	24.0-26.0	16	95	65	29-300	49		—				--		
0.28	6.5	0.8	4.7-8.4	50	7.5	0.4	6.9-8.7	51	25.4	0.6	24.0-26.5	18	95	65	29-310	49		—				--		
0.62	6.6	0.8	4.8-8.5	50	7.5	0.4	6.9-8.7	51	25.2	0.3	25.0-26.0	14	94	64	29-301	49		--				--		
1.36	7.1	0.6	5.7-8.8	50	7.5	0.4	6.9-8.6	51	25.1	0.4	24.5-26.0	13	94	64	29-298	49		--				--		

Daphnia magna

TNT. The effect of TNT on daphnid survival is shown in Table 28. No apparent toxicant-related increase in mortality occurred within the range of concentrations tested (0.03 to 1.03 mg/L).

Table 28. CUMULATIVE MORTALITY OF DAPHNIDS DURING A 28-DAY EXPOSURE TO TNT

Average Actual Concentration (mg/L)	Number Dead (n = 22)					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
Control	1	2	2	1	1	1
0.03	1	1	1	1	2	3
0.08	0	0	0	0	1	2
0.24	0	1	1	1	1	1
0.48	4	4	6	1	2	2
1.03	0	0	0	1	1	1

The effect of TNT on reproductive success in daphnids is shown in Table 29. The data indicate that young production in Series A was reduced at the highest concentration (1.03 mg/L) at exposure periods of 14 and 21 days. Because cumulative reproduction at the highest concentration exceeded that in the controls by Day 28, this effect could be considered transitory. On the other hand, survival of daphnids in the real world is likely to be less than 28 days so the impaired reproduction could be of biological significance.

Data on the onset of reproduction, the number of young produced per day during the reproductive period and the length of surviving females are shown in Table 30. Although quite variable, the time to the onset of reproduction showed no trends related to the presence of TNT. Daily young production was significantly reduced in the pooled series at 0.03 and 0.08 mg/L and in Series B alone at 0.08 mg/L. However, in view of the nonsignificant responses at the higher concentrations (0.24 to 1.03 mg/L), it is unlikely that the responses at 0.03 and 0.08 mg/L were toxicant-related. TNT did not appear to affect the length of daphnids after 28 days of exposure.

Chemical analyses associated with this test are summarized in Table 31. The measured concentrations agreed well with the nominal concentrations, except that the two lowest concentrations were 50 to 75% of their nominal values. This result was most likely due either to continuous adsorption or to photolysis of TNT.

Table 29. AVERAGE NUMBER OF YOUNG PRODUCED BY INDIVIDUAL DAPHNIDS EXPOSED TO TNT

Average Actual Concentration (mg/L)	Number of Young Produced					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
Control	8.7	34.3	64.4	6.4	43.7	98.6
0.03	4.6	29.0	59.3	7.5	42.7	68.3
0.08	7.6	35.6	61.0	9.0	44.4	73.7
0.24	4.4	34.7	79.7	5.4	29.4	72.9
0.48	7.2	36.7	82.8	10.6	39.7	78.0
1.03	2.7[a]	22.1[a]	97.9	2.8	36.2	88.2

[a]Statistically significant, $p < 0.05$.

Table 30. TIME TO FIRST BROOD, NUMBER OF YOUNG PRODUCED PER REPRODUCTIVE DAY, AND LENGTH OF SURVIVING DAPHNIDS EXPOSED TO TNT FOR 28 DAYS

Average Actual Concentration (mg/L)	Test Series	Time to First Brood (days)	No. Young Produced per Day	Average Length (mm)
Control	A	11.9	4.0	3.8
	B	12.6	6.3	3.9
0.03	A	11.9	3.7	3.7
	B	12.3	4.4	3.8
0.08	A	11.3	3.6	3.9
	B	11.1	4.3[a]	4.0
0.24	A	11.6	4.8	3.8
	B	18.6	7.8	3.5
0.48	A	11.7	5.2	4.2
	B	13.1	4.7	3.8
1.03	A	13.3	6.6	4.2
	B	14.0	6.3	4.0

[a]Statistically significant, $p < 0.05$.

Table 31. TNT CONCENTRATIONS OBTAINED DURING THE DAPHNID CHRONIC STUDY ON TNT

Nominal Concentration (mg/L)	Analyzed Concentration (mg/L)			
	x	S.D.	Range	n
Control	0	—	—	7
0.06	0.03	0.022	0.00-0.06	7
0.12	0.08	0.030	0.03-0.12	7
0.25	0.24	0.111	0.16-0.48	7
0.50	0.48	0.130	0.28-0.67	7
1.00	1.03	0.263	0.60-1.38	7
Stock	94.1	20.03	74.8-114.8	3
Spike[a]	10.7	2.17	8.2-12.1	3

[a]Nominally 10% of stock.

LAP water. Mortality of daphnids exposed to LAP is shown in Table 32. Survival was significantly affected in Series A at 0.03 and 0.31 mg/L after 28 days of exposure. The reduction in survival was also significant in the pooled series at 0.31 mg/L after 21 and 28 days of exposure. In spite of these significant responses, the lack of any apparent concentration-related trend and, in particular, the lack of any effect on mortality at the highest test concentration suggest that the observed responses were not toxicant-related.

Table 32. CUMULATIVE MORTALITY OF DAPHNIDS DURING A 28-DAY EXPOSURE TO LAP WATER

Average Measured Concentration (mg/L)	Number Dead (n = 22)					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
Control	0	0	0	2	3	4
0.03	3	5	8[a]	1	1	3
0.04	0	0	0	9	10	10
0.13	1	1	2	5	8	9
0.31	5	6[b]	8[a]	1	11[b]	12[b]
0.72	0	0	0	1	1	2

[a]Statistically significant, $p < 0.05$.
[b]Statistically significant, $p < 0.05$, if series are pooled.

The effect of LAP water on reproductive success in daphnids is shown in Table 33. In Series A, young production was significantly reduced at concentrations of 0.03 to 0.31 mg/L after 14 days of exposure and at 0.04 to 0.31 mg/L after 21 and 28 days of exposure. In Series B, the only significant reduction in the number of young produced occurred at 0.04 mg/L after 14 days of exposure. If the series were pooled, the effect was significant at 0.03 to 0.04 mg/L after 14 days of exposure, at 0.03 mg/L after 21 days of exposure, and at 0.13 mg/L after 28 days of exposure. The lack of significant effects at the highest concentration in both series suggests that the observed reductions in young production at lower concentrations may not be a direct consequence of the toxicant. This argument is particularly plausible for Series B, where young production at the highest concentration was consistently higher than that of the controls. However, in Series A, production at all concentrations was lower than that of the controls, averaging 24% less in all exposure periods at the highest concentration (0.72 mg/L).

Table 33. AVERAGE NUMBER OF YOUNG PRODUCED BY INDIVIDUAL DAPHNIDS EXPOSED TO LAP WATER

Average Actual Concentration (mg/L)	Number of Young Produced					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
Control	78.6	180.3	237.1	24.3	59.4	113.4
0.03	45.4[a]	119.8	179.3	16.5	43.8	106.2
0.04	40.0[a]	104.0[a]	146.7[a]	4.7[a]	56.5	98.5
0.13	33.4[a]	89.6[a]	154.0[a]	25.4	69.2	106.6
0.31	33.4[a]	90.5[a]	145.0[a]	23.9	84.9	130.0
0.72	58.6	125.3	194.6	32.0	96.2	167.3

[a]Statistically significant, $p < 0.05$.

The effect of LAP water on the time to first brood, number of young produced per day during the reproductive period, and the length of adult daphnids is shown in Table 34. The time to first brood did not appear to be affected by LAP water within the range of concentrations tested (0.03 to 0.72 mg/L). The number of young produced per reproductive day was significantly reduced in Series A at 0.31 mg/L and in the pooled series at 0.04 and 0.13 mg/L. The average length of individual adult daphnids was reduced in Series A at 0.04 and 0.13 mg/L and in the pooled series at 0.04, 0.13, and 0.31 mg/L. As with survival and young production, the lack of any significant response at the highest concentration (0.72 mg/L), suggests that the observed responses were not due to the toxic action of LAP water.

The apparent lack of a significant effect on any of the test parameters at the highest concentration and the presence of effects at lower concentrations suggest that there was an interaction between the toxicant and some other variable associated with the test. Several possible explanations for what may have occurred exist. LAP water, or related breakdown products, may have been used as a food substrate for microbial growth, which, in turn, would have increased the food available for the daphnids. This theory may have some merit based on the reproduction and growth data. In Series B, reproduction was higher at the two highest concentrations (0.31 and 0.72 mg/L), and growth was similar to or greater than in the controls. These daphnids were fed algae supplemented by a daphnid formula that incorporated trout chow and yeast (Biesinger and Christensen, 1972). In series A, where the daphnids were fed algae plus a vitamin supplement (Goulden et al., 1982), reproduction and growth were greater in the controls than in daphnids at all concentrations. The algae-plus-vitamin diet appeared to be more nutritious because daphnids on this diet had markedly higher reproduction and somewhat better growth than daphnids fed algae and the trout food supplement.

Table 34. TIME TO FIRST BROOD, NUMBER OF YOUNG PRODUCED PER REPRODUCTIVE DAY, AND LENGTH OF SURVIVING DAPHNIDS EXPOSED TO LAP WATER FOR 28 DAYS

Average Measured Concentration (mg/L)	Test Series	Time to First Brood (days)	No. Young Produced per Day	Average Length (mm)
Control	A	8.6	12.2	4.6
	B	8.4	5.8	4.2
0.03	A	9.0	10.2	4.3
	B	11.3	6.9	4.1
0.04	A	9.0	7.7	3.9[a]
	B	8.0	4.5	3.8
0.13	A	9.0	8.1	4.0[a]
	B	8.5	5.2	3.9
0.31	A	9.0	7.7[a]	4.2
	B	10.4	7.2	4.0
0.72	A	9.0	10.2	4.4
	B	10.0	9.41	4.3

[a]Statistically significant, $p < 0.05$.

Another possible explanation is that LAP water may have elicited a toxic effect on other organisms in the beakers that could have competed with the daphnids. We did observe that the occurrence of small, white nematode-like worms in some of the beakers appeared to be related to the mortality of daphnids in those beakers. In fact, we were forced to restart one of the series due to excessive mortality. However, in the restarted Series A, the worms were also present at the highest concentration and no mortality occurred.

If diet was less of a limiting factor in Series A than in Series B, then it would appear that chronic exposure to LAP water within a concentration range of 0.03 to 0.72 mg/L had a deleterious, although not always statistically significant, effect on daphnids. This is based on the observed reductions in reproduction and growth parameters at these concentrations compared with the controls. Similarly, the growth and reproduction responses observed in Series B are suggestive of effects at the lower concentrations (0.03 to 0.04 mg/L) with some compensatory mechanism(s) operating at the higher concentrations (0.13 to 0.72 mg/L). We suggest that this test be repeated before drawing any conclusions about long-term sensitivity of D. magna to LAP water.

The results of chemical analyses associated with this study are summarized in Table 35. We have no explanation for the low measured level of the nominal 0.12 mg/L concentration. This level also exhibited the lowest ratio of TNT to RDX, which indicates that TNT was being lost at a faster rate than at the other concentrations. However, this result cannot be explained on the basis of diluter malfunction or by differences between the test series.

Table 35. LAP WATER CONCENTRATIONS OBTAINED DURING THE DAPHNID CHRONIC STUDY

Nominal Concentration (mg/L)	Analyzed Concentration (mg/L)				Ratio of TNT:RDX
	$\bar{x}$	S.D.	Range	n	
Control	0	—	—	8	--
0.06	0.03	0.014	0.00-0.04	8	1.0
0.12	0.04	0.021	0.01-0.06	8	0.8
0.25	0.13	0.026	0.08-0.17	8	1.2
0.50	0.31	0.092	0.20-0.45	8	1.2
1.00	0.72	0.185	0.45-0.95	8	1.2
Stock	125	13.8	100-149	7	1.4
Spike[a]	11.1	1.15	10.4-12.2	7	1.4

[a]Nominally 10% of stock.

LAP-Irrad. The effect of LAP-Irrad on daphnid survival is summarized in Table 36. There were no statistically significant effects on survival

within the range of concentrations tested (0.01 to 0.18 mg/L, based on the amount of TNT and RDX present after photolysis). The overall mortality response did not appear to be toxicant-related and exhibited the same characteristics--greater number of deaths at the intermediate concentrations than in the controls or at the highest concentration--seen in the test on LAP.

Table 36. CUMULATIVE MORTALITY OF DAPHNIDS EXPOSED TO LAP-IRRAD WATER FOR 28 DAYS

Nominal Concentration[a] (mg/L)	Number Dead (n = 22)					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
Control	3	3	3	4	4	4
0.07	4	4	4	4	4	5
0.14	5	5	5	3	3	5
0.28	4	4	5	8	9	10
0.76	5	8	10	5	6	6
1.25	2	2	4	3	3	3

[a]Prior to photolysis; see Table 39

Data on reproduction in daphnids exposed to LAP-Irrad are shown in Table 37. In Series A, reproduction did not appear to be affected except at the highest concentration (0.18 mg/L) at 21 and 28 days of exposure. This effect was significant at 28 days. In Series B, it appears that there may have been a toxicant-related reduction in the number of young produced at the two highest concentrations (0.11 and 0.18 mg/L) after 21 and 28 days of exposure. This effect was significant at 0.11 mg/L after 21 and 28 days. If the results from both series are pooled, young production was significantly reduced at the highest concentration (0.18 mg/L) after 28 days of exposure.

Table 37. AVERAGE NUMBER OF YOUNG PRODUCED BY INDIVIDUAL DAPHNIDS EXPOSED TO LAP-IRRAD

Nominal Concentration[b] (mg/L)	Number of Young Produced					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
Control	35.0	98.9	187.6	40.3	117.0	172.0
0.07	40.0	105.2	188.2	27.6	96.0	148.6
0.14	32.5	96.2	159.5	37.6	128.0	168.0
0.28	47.5	135.0	192.5	47.4	144.0	186.4
0.76	50.7	134.6	190.8	30.9	87.7[a]	130.6[a]
1.25	40.3	85.5	126.3[a]	38.0	103.3	141.4

[a]Statistically significant, $p < 0.05$.
[b]Prior to photolysis; see Table 39.

The effect of LAP-Irrad on time to first brood, number of young produced per reproductive day, and the length of adult daphnids is shown in Table 38. There appeared to be no toxicant-related effects on the time to first brood or on the length of adult daphnids. The number of young produced per reproductive day was significantly reduced in Series A at 0.18 mg/L, in Series B at 0.11 mg/L, and in the pooled series at 0.18 mg/L.

Chemical analyses associated with the test on LAP-Irrad are summarized in Table 39. The analytical results are based on the actual amount of TNT and RDX that remained after photolysis. In addition, the corresponding initial concentrations of LAP water, prior to photolysis and based on a stock concentration of 60 mg/L LAP water, are also presented.

Water quality associated with the daphnid chronic studies on TNT, LAP water and LAP-Irrad are summarized in Tables 40-42.

Table 38. TIME TO FIRST BROOD, NUMBER OF YOUNG PRODUCED PER REPRODUCTIVE DAY, AND LENGTH OF SURVIVING DAPHNIDS EXPOSED TO LAP-IRRAD FOR 28 DAYS

Nominal Concentration (mg/L)[b]	Test Series	Time to First Brood (days)	No. Young Produced per Day	Average Length (mm)
Control	A	10.4	10.7	4.3
	B	12.0	10.8	4.3
0.07	A	9.6	10.2	4.5
	B	12.8	9.9	4.5
0.14	A	12.0	9.8	4.3
	B	10.2	9.8	4.3
0.28	A	10.0	10.7	4.4
	B	11.4	11.2	4.5
0.76	A	10.4	11.2	4.5
	B	11.4	7.9[a]	4.3
1.25	A	10.3	7.3[a]	4.4
	B	11.9	8.7	4.4

[a]Statistically significant, $p < 0.05$.

[b]Prior to photolysis; see Table 39.

Table 39. LAP-IRRAD CHEMICAL CONCENTRATIONS OBTAINED DURING THE DAPHNID CHRONIC STUDY

Nominal Concentration (mg/L)[a]	Analyzed Concentration (mg/L) $\bar{x}$	S.D.	Range	n
Control	0	--	--	4
0.01	0.01	0	--	4
0.03	0.02	0.005	0.01-0.02	4
0.06	0.04	0.006	0.04-0.05	4
0.12	0.11	0.025	0.08-0.14	4
0.25	0.18	0.039	0.15-0.24	4
Stock	8.64	2.354	6.97-10.30	2
Spike[b]	0.76	0.085	0.70-0.82	2

[a]These concentrations correspond to 0.00, 0.07, 0.14, 0.28, 0.76, and 1.25 mg/L as unphotolyzed LAP.

[b]Nominally 10% of stock.

Table 40. WATER QUALITY PARAMETERS MONITORED DURING THE DAPHNID CHRONIC STUDY WITH TNT

Concentration	Dissolved oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
(mg/L)	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n
Control	8.4	0.3	8.0-8.6	4	8.4	0.1	8.3-8.6	4	21.6	0.25	21.3-21.9	15	149	32	119-179	4	44	12	18-57	13	33	12	23-49	4
0.03	8.4	0.3	7.9-8.6	4	8.4	0.2	8.2-8.6	4	21.2	0.5	21.0-22.0	4	145	31	119-185	4		--				--		
0.08	8.4	0.3	7.9-8.6	4	8.3	0.2	8.0-8.6	4	21.2	0.5	21.0-22.0	4	139	31	115-181	4		--				--		
0.24	8.3	0.1	7.9-8.5	4	8.3	0.3	7.9-8.6	4	21.2	0.5	21.0-22.0	4	131	30	109-170	4		--				--		
0.48	8.4	0.2	8.2-8.6	4	8.3	0.2	8.1-8.6	4	21.2	0.5	21.0-22.0	4	118	29	99-159	4		--				--		
1.03	8.4	0.2	8.2-8.6	4	8.3	0.3	8.0-8.7	4	21.2	0.5	21.0-22.0	4	114	28	98-149	4		--				--		

Table 41. WATER QUALITY PARAMETERS MONITORED DURING THE DAPHNID CHRONIC STUDY WITH LAP WATER

Concentration	Dissolved oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
(mg/L)	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n
Control	8.6	0.3	8.1-9.1	8	8.2	0.4	7.3-8.7	8	20.6	0.4	20.0-21.0	8	136	22	115-165	5	44	14	28-94	17	28	4	21-33	8
0.03	8.6	0.3	8.1-9.1	8	8.1	0.3	7.4-8.4	8	20.6	0.4	20.0-21.0	8	134	20	115-160	5		--				--		
0.04	8.5	0.4	8.0-9.1	8	8.2	0.3	7.5-8.5	8	20.6	0.4	20.0-21.0	8	131	22	111-160	5		--				--		
0.13	8.5	0.3	8.1-8.9	8	8.2	0.3	7.6-8.4	8	20.6	0.4	20.0-21.0	8	124	20	105-150	5		--				--		
0.31	8.5	0.3	8.0-8.9	8	8.2	0.3	7.6-8.5	8	20.6	0.4	20.0-21.0	8	113	14	98-130	5		--				--		
0.72	8.6	0.3	8.1-8.9	8	8.1	0.3	7.6-8.4	8	20.6	0.4	20.0-21.0	8	102	14	85-120	5		--				--		

Table 42. WATER QUALITY PARAMETERS MONITORED DURING THE DAPHNID CHRONIC STUDY WITH LAP IRRAD

Concentration	Dissolved oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
(mg/L)	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n	Mean	S.D.	Range	n
Control	8.6	0.3	8.2-8.9	4	7.9	0.2	7.7-8.1	4	20.0	0.3	19.0-20.0	14	212	11	205-220	2	69	20	40-100	14	63	4	60-66	2
0.01	8.6	0.3	8.2-8.8	4	8.0	0.2	7.7-8.1	4	20.0	2.0	19.0-21.0	3	222	25	205-240	2		--				--		
0.02	8.5	0.3	8.2-8.8	4	7.9	0.2	7.6-8.1	4	20.0	0.6	19.0-20.0	3	222	25	205-240	2		--				--		
0.04	8.5	0.2	8.2-8.7	4	8.0	0.2	7.7-8.2	4	20.0	1.0	19.0-21.0	3	210	14	200-220	2		--				--		
0.11	8.4	0.2	8.2-8.6	4	7.8	0.2	7.6-8.1	4	20.0	0.6	19.0-20.0	3	195	7	190-200	2		--				--		
0.18	8.4	0.2	8.2-8.6	4	7.8	0.2	7.6-8.1	4	20.0	1.0	19.0-21.0	3	188	4	185-190	2		--				--		

Calculation of Water Quality Criteria

The primary purpose for developing an aquatic toxicity data base on 2,4,6-TNT and LAP water is to establish water quality criteria for these materials. The following procedure is taken from EPA (1980). The criteria consists of two components. The first is the maximum allowable concentration, which is the Final Acute Value, as calculated from the results of 48- and 96-hour acute studies. The second criterion is the maximum 24-hour average concentration, which is the lowest of the following values: the Final Chronic Value, the Final Plant Value, and the Final Residue Value.

2,4,6-TNT

Final Acute Value. The data base (Liu et al., 1984) used to derive the Final Acute Value for TNT is shown in Table 43. Application of the EPA procedure gives a Final Acute Value of 0.9 mg/L. This value is slightly higher than one of acute values obtained for rainbow trout and is also higher than the incipient lethal concentration determined for daphnids (0.19 mg/L) during a 2-week flow-through test (Liu et al., 1984). However, given the fact that the criteria contains a maximum 24-hour average component, 0.9 mg/L appears to be a reasonable estimate of the maximum allowable concentration, at least on an interim basis until other data prove differently.

Final Chronic Value. The data used to calculate the Final Chronic Value for TNT are summarized in Table 44. Acute/chronic toxicity ratios were calculated using the 96-hour LC50 estimates from flow-through tests on fathead minnows and rainbow trout and the 48-hour LC50 estimate from the flow-through test on D. magna divided by the appropriate geometric means from Table 44. Two of these values were estimates; 0.04 mg/L was used for the geometric mean of the fathead minnow chronic study even though effects occurred at this concentration and 4.4 mg/L was used as the 48-hour value for D. magna although this concentration did not kill 50% of the exposed organisms within 48 hours in the flow-through test. The geometric mean of these three values was then calculated. This average acute/chronic ratio--18.6--was then divided into the Final Acute Value--0.9 mg/L--to obtain a Final Chronic Value of 0.05 mg/L. However, this value may not be adequate to protect aquatic organisms from effects of chronic exposure to TNT because effects of TNT on reproduction and the F_1 generation were seen in the chronic test on fathead minnows at the lowest concentration tested, 0.04 mg/L. Therefore, the Final Chronic Value should be at least as low as 0.04 mg/L.

Final Plant Value. The Final Plant Value for TNT is the lowest concentration that significantly affected growth. Data from the 14-day algal assays presented in Volume I (Liu et al., 1984) indicate that Microcystis aeruginosa, Selenastrum capricornatum, and Anabaena flos-aquae were the most sensitive algal species, with effects occurring at concentrations as low as 4.1 mg/L.

Table 43. SUMMARY OF ACUTE TOXICITY DATA--LC50 VALUES (mg/L) FOR TNT

Bluegill	Fathead Minnow	Channel Catfish	Rainbow Trout	Daphnia magna	Lumbriculus variegatus	Hyallela azteca	Paratanytarsus dissimiis
3.4	1.2	2.4	1.5	11.7	5.2	6.5	27.0
2.6	2.1	3.3	0.8	> 4.4	> 29.0		
2.5	2.4		2.0				
	2.9						
	3.7						
Geometric Mean							
2.8	2.3	2.8	1.3	11.7	5.2	6.5	27.0

Table 44. DATA BASE FOR FRESHWATER CHRONIC VALUE—TNT

Species	Test Type	Response Parameter	MATC Limits (mg/L)	Geometric Mean (mg/L)
Rainbow trout	Early life stage	Fry survival	0.13-0.24	0.18
Fathead minnow	Early life stage[a]	Fry survival and growth	0.42-0.84	0.35
	Chronic	Reproduction, F_1 Hatching success, Survival and growth	< 0.04[b]	None
Channel catfish	Early life stage[a]	Hatching success, Fry survival	> 1.35	None
Daphnia magna	Chronic	Reproduction	0.48-1.03	0.70

[a] Poor tests; included for informational purposes and not used in calculations.
[b] Value of 0.04 used in calculations.

Final Residue Value. The Final Residue Value (FRV) is calculated using bioconcentration data and the lowest published lethal dose from the Registry of Toxic Effects of Chemical Substances (Lewis and Tathen, 1982). In this case, data from the muscle tissue of bluegills exposed to TNT for 96 hours resulted in an estimated BCF of 9.5 (Liu et al., 1983). Because these data were obtained from a relatively short exposure period, we also calculated the BCF using the log P estimate for TNT (2.03) and the equation recommended by the EPA (1980) for estimating the steady-state BCF for organic compounds in organisms that contain about 8% lipids. This approach results in an estimated BCF of 20.5. Because of the short exposure period used in the bioconcentration experiment on TNT, we elected to follow a conservative approach in estimating the FRV and used the calculated value of 20.5 as the BCF estimate. The FRV is the oral LD50 for rabbits exposed to TNT--500 mg/kg--divided by the BCF--20.5, i.e., 24.4 mg/L.

Water Quality Criteria. The maximum allowable concentration of TNT is the Final Acute Value--0.9 mg/L. The 24-hour average concentration is the lowest value selected from the Final Chronic Value, the Final Plant Value, and the Final Residue Value. In this case, the Final Chronic Value--0.04 mg/L--is the lowest value and is therefore the allowable 24-hour average concentration. This is actually a fairly rigorous standard as TNT could only be discharged at the 0.9 mg/L level for 64 minutes per day followed by zero discharge, in order to meet the 24-hour average criteria. Caution should be used, however, when applying these criteria because some chronic effects were seen at 0.04 mg/L TNT.

LAP Water

Final Acute Value. The data (Liu et al., 1984) used to derive the Final Acute Value for LAP water are summarized in Table 45. Application of the EPA procedure gives a value of 1.3 mg/L. This value is lower than any of the incipient LC50s obtained (Liu et al., 1984). Given that the criteria contains a 24-hr average concentration in addition to the maximum allowable concentration, 1.3 mg/L should provide reasonable protection from acute effects.

Final Chronic Value. The data available to calculate the Final Chronic Value for LAP water are summarized in Table 46. The guidelines for calculating water quality criteria specify that chronic data from a mimimum of three species must be used to determine the Final Chronic Value. This criterion is not satisfied because relatively poor survival of control fry in the early life stage tests and ambiguous results in the daphnid chronic study rendered these tests unacceptable for use in setting a water quality criteria. Therefore, in order to provide some estimate of a Final Chronic Value for LAP water, we used only the results of the fathead minnow chronic study. These results were ambiguous in that the F_1 growth appeared affected at 0.11 mg/L in fry reared to 30 days but not in fry reared to 60 days. Consequently, we used 0.28 and 0.62 mg/L, respectively, as the no-effect-effect levels based on F_0 survival. The geometric mean of these values, 0.42 mg/L, was divided into the geometric mean for fathead minnows from Table 45 to obtain an acute-chronic toxicity

ratio--6.67. This value, divided into the final acute value--1.3 mg/L gives a Final Chronic Value of 0.19 mg/L.

Final Plant Value. Microcystis aeruginosa was the most sensitive algal species tested (Liu et al., 1984). The lowest concentration of LAP water that caused significant effects on growth was 0.6 mg/L, which is therefore the Final Plant Value.

Final Residue Value. Calculating a freshwater FRV for LAP water is somewhat problematical because of limited data on bioconcentration. Liu and co-workers (1983) reported a 4-day BCF of 3.9 in bluegill muscle tissue for LAP water, and values of 1.9 and 9.5, respectively, for RDX and TNT alone. Bentley et al. (1978) found 3- and 28-day BCFs for RDX of 9.5 and 3.7, respectively, in bluegill muscle tissue. Because of the disparities in the exposure periods and BCF estimates, we again elected to take the conservative approach and use the calculated BCF for TNT (20.5) in our calculations of the FRV. The FRV for LAP water is the oral LDLO for Fischer-344 rats--150 mg/kg (Brown et al., 1983)--divided by the BCF for TNT, or, 150 mg/kg ÷ 20.5 = 7.3 mg/L.

Water Quality Criteria. The maximum allowable concentration of LAP water is the Final Acute Value--1.3 mg/L. The allowable 24-hour average concentration is the lowest value selected from the Final Chronic Value, the Final Plant Value, and the Final Residue Value. In this case, the final Chronic Value--0.19 mg/L--is the lowest value and should be considered as the allowable 24-hour concentration. In order for the average concentration to be met, LAP water could only be discharged between three and four hours per day at 2.3 mg/L with no discharge taking place the rest of the day.

Table 45. SUMMARY OF ACUTE TOXICITY DATA--LC50 VALUES (mg/L) FOR LAP WATER

Bluegill	Fathead Minnow	Channel Catfish	Rainbow Trout	Daphnia magna	Lumbriculus variegatus	Hyallela azteca	Paratanytarsus dissimiis
4.2	5.9	5.2	1.7	11.6	9.0	10.4	31.8
3.2	1.1	4.5	1.5	> 6.8	27.4		
2.5	0.7						
4.2	4.2						
2.5	5.1						
3.2	4.8						
2.9							
2.4							
2.5							
2.9							
3.4							
Geometric Mean							
3.0	2.8	4.8	1.6	11.6	15.7	10.4	31.8

Table 46. DATA BASE FOR FRESHWATER FISH CHRONIC VALUE--LAP WATER

Species	Test Type	Response Parameter	MATC Limits (mg/L)	Geometric Mean (mg/L)
Rainbow trout	Early life stage[a]	Growth	0.21-0.45	0.31
Fathead minnow	Early life stage[a]	Survival	0.53-1.02	0.74
	Chronic	F_0 survival	0.28-0.62	0.42
Channel catfish	Early life stage[a]	Survival	0.88-1.96	1.31

[a] Poor tests; included for informational purposes and not used in calculations.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Based on the data presented in this report, we conclude that:

- TNT is toxic to fathead minnows, the most sensitive of the organisms tested, at a concentration of 0.04 mg/L. This toxicity is manifested primarily in reproductive effects and effects on F_1 survival and growth.

- LAP water is definitely toxic to fathead minnows at 0.62 mg/L based on F_0 survival. There are suggestions of effects at 0.11 mg/L in F_1 growth and 0.05 mg/L on the global indices in one of the test series.

- Based on initial concentrations of TNT and RDX, photolyzed LAP water was less toxic than LAP water to D. magna under conditions of chronic exposure. This is in agreement with the results of the acute studies on photolyzed and nonphotolyzed solutions reported in Volume I.

Recommendations

- Additional studies should be performed to determine a no-effect level for fathead minnows exposed to TNT and for D. magna exposed to LAP.

- A concentration of 0.9 mg/L TNT should be used as the "maximum allowable concentration in a 24-hour period" component of the water quality criterion for TNT.

- A concentration of 0.04 mg/L TNT should be used as an interim "24-hour average allowable concentration" component of the water quality criterion for TNT until a no-effect concentration is obtained for fathead minnows exposed to this material.

- A concentration of 1.3 mg/L LAP should be used as the "maximum allowable concentration in a 24-hour period" component of the water quality criterion for LAP.

- A concentration of 0.19 mg/L LAP should be considered as the "24-hour average allowable concentration" component of the water quality criterion for LAP.

- Given the suggestion of chronic effects below levels that produced statistically significant differences from the controls and the fact that the LAP standard is based on a synthetic wastewater, additional work should be considered to assure the appropriateness of the criteria. Field monitoring at discharge sites would aid in determining the occurrence of effects on local populations of organisms. Given the observed effects on reproduction and the F_1 generation, such monitoring should be on a long-term basis as effects may not be apparent for several generations. Additional bioassays that focus on concentrations at, and below, the criteria would also aid in determining the presence of effects at these levels.

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